

AD _____

Award Number: DAMD17-99-1-9013

TITLE: Evaluation of Immunotoxicity Due to Concurrent Exposure to
DEET, Pyridostigmine, and JP-8 Jet Fuel

PRINCIPAL INVESTIGATOR: Deborah E. Keil, Ph.D.

CONTRACTING ORGANIZATION: Medical University of South Carolina
Charleston, South Carolina 29425

REPORT DATE: March 2000

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for public release;
distribution unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

20000508 154

REPORT DOCUMENTATION PAGEForm Approved
OMB No. 074-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503

1. AGENCY USE ONLY (Leave blank)		2. REPORT DATE March 2000	3. REPORT TYPE AND DATES COVERED Annual (1 Feb 99 - 31 Jan 00)	
4. TITLE AND SUBTITLE Evaluation of Immunotoxicity Due to Concurrent Exposure to DEET, Pyridostigmine, and JP-8 Jet Fuel			5. FUNDING NUMBERS DAMD17-99-1-9013	
6. AUTHOR(S) Deborah E. Keil, Ph.D.				
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Medical University of South Carolina Charleston, South Carolina 29425 E-MAIL: keilde@musc.edu			8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012			10. SPONSORING / MONITORING AGENCY REPORT NUMBER	
11. SUPPLEMENTARY NOTES Report contains color graphs				
12a. DISTRIBUTION / AVAILABILITY STATEMENT Approved for public release; distribution unlimited				12b. DISTRIBUTION CODE
13. ABSTRACT (Maximum 200 Words) Military personnel during the Gulf War were exposed to various compounds including <i>N,N</i> -diethyl- <i>m</i> -toluamide (DEET), pyridostigmine bromide (PYR), and JP-8 jet fuel. The effects of concurrent exposure to these agents could potentially result in significant health effects or contribute to the manifestations of Gulf War Syndrome. Adenopathy, fever, joint pain, and skin rash are reported by veterans and these signs may be linked to immune dysfunction. Recent studies indicate that neurotoxicity increases during concurrent exposure to both PYR and DEET with other agents. Due to known interdependencies of the immune and nervous systems, it is hypothesized that the effects of PYR and DEET may affect the immune system. Additionally, single low-level exposures to JP-8 have been reported to significantly affect T- and B-lymphocyte populations. Thus, the possibility exists that combinations of DEET and PYR could exacerbate the effects of JP-8 or cause a predisposition to other immune conditions such as autoimmune disease. Therefore, this investigation will evaluate the effects of concurrent exposure to these common military agents on immunological parameters (i.e., natural killer cell activity, macrophage function, etc.) and susceptibility to diseases such as cancer, infections, and autoimmune disease.				
14. SUBJECT TERMS Gulf War				15. NUMBER OF PAGES 59
				16. PRICE CODE
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unlimited	

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)
Prescribed by ANSI Std. Z39-18
298-102

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

DK Where copyrighted material is quoted, permission has been obtained to use such material.

DK Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

DK Citations of commercial organizations and trade names in this report do not constitute an official Department of Army endorsement or approval of the products or services of these organizations.

X In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and use of Laboratory Animals of the Institute of Laboratory Resources, national Research Council (NIH Publication No. 86-23, Revised 1985).

N/A For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

N/A In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

N/A In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

N/A In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

Debra Keil
PI - Signature

Feb 28-2000
Date

TABLE OF CONTENTS

	Page
COVER PAGE	i
STANDARD FORM (SF) 298: REPORT DOCUMENTATION PAGE	ii
FOREWORD	iii
TABLE OF CONTENTS	1
INTRODUCTION	2
BODY	3
Progress Toward Objectives/Tasks	3
Preliminary Results and Discussion	5
Range Finding Studies	6
JP-8 Jet Fuel Single Exposure Studies	6
DEET Single Exposure Studies	7
Pyridostigmine Bromide Single Exposure Studies	9
Concurrent JP-8, DEET, & PYR Exposure Studies (LOIEL).	10
KEY RESEARCH ACCOMPLISHMENTS	11
REPORTABLE OUTCOMES	12
CONCLUSIONS	14
REFERENCES	16
APPENDIX A: FIGURES AND GRAPHS	18
APPENDIX B: SUBMITTED ABSTRACTS	44
APPENDIX C: CURRICULUM VITAE	48

INTRODUCTION

Approximately 700,000 U.S. service personnel were involved in the sea, air, and ground war in the Persian Gulf from August 1990 to April 1991. Upon their return from the Gulf War, some veterans reported a variety of manifestations such as muscle fatigue, myalgia, impaired cognition, ataxia, headaches, adenopathy, fever, joint pain, skin rash, gastrointestinal disturbances, respiratory difficulties, and gynecologic problems (1,2,3,4). However, the precise cause(s) of these manifestations has remained an enigma. While on this duty, service personnel were concurrently exposed to a variety of environmental chemicals and stressful events. *N,N*-diethyl-*m*-toluamide (DEET), pyridostigmine bromide (PYR), and JP-8 jet fuel were used throughout the duration of the war. It is not known whether concurrent exposure to these agents would predispose veterans to disease or contribute to the manifestations experienced by veterans. While independent exposure to PYR or DEET has been reported to cause minimal physiological effects in humans, recent studies have shown that concurrent exposure to PYR and DEET, with or without stress induction or additional agents, increased neurotoxicity or lethality (5,6,7,8, 9,10,11). Additionally, it was recently reported that exposure to JP-8 causes significant immunotoxicological effects (12). Potential effects to immunological function, however, were not determined for combined exposures to DEET, PYR, and JP-8. Since these agents were part of the Persian Gulf environment and there are unexplained immune manifestations in Gulf War veterans, it is rationale to determine potential effects on immune function and susceptibility to cancer and infectious or autoimmune disease caused by concurrent PYR, JP-8, and DEET exposure. Ultimately, this may provide explanations to the variety of manifestations experienced by Gulf War veterans or indicate potential health risks for future soldiers exposed to a similar regimen.

PROGRESS TOWARD OBJECTIVES/TASKS

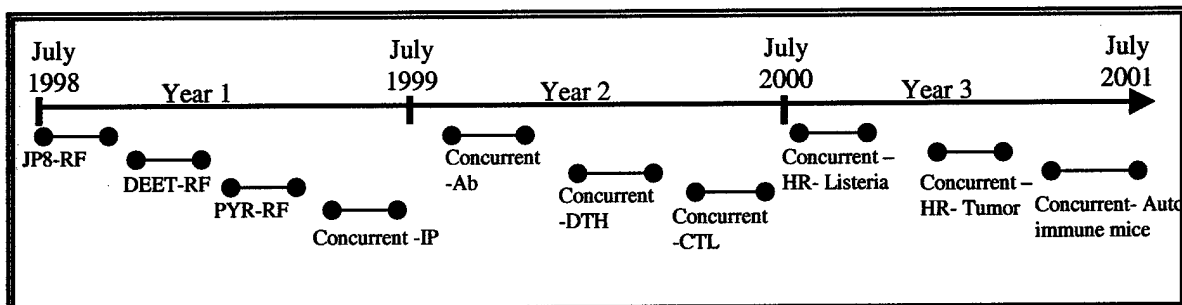


Figure 1. Original Timetable for the Achievement of the Specific Objectives:

Year 1. Specific Aim #1: Range finding studies and evaluation of immune parameters following individual exposures to DEET, PYR, or JP-8 will be performed. Concurrent exposure to DEET, PYR, and JP-8 will begin.

Year 2. Specific Aim #1: Immunotoxic effects of the combined exposure to DEET, PYR, and JP-8 will be evaluated. Specific Aim #2 will begin: Host resistance to bacteria and tumor challenges after concurrent exposure to DEET, PYR, and JP-8.

Year 3. Specific Aim #2: Host Resistance Models will be completed. Specific Aim #3: Possible induction of autoimmune diseases will be completed. Susceptibility to disease due to the combined exposure of DEET, PYR, and JP-8 will be assessed. Final reports and manuscripts will be completed.

Legend: RF= Range finding studies, IP= immune parameters, Ab=Antibody assessment, DTH=Delayed-type hypersensitivity, CTL= Cytotoxic T cell activity, HR= Host Resistance challenges

Year 1 and 2- Specific Aim (Objective) 1: To test the hypothesis that 14-day concurrent exposure to low-levels of DEET, PYR and JP-8 will significantly affect a variety of immunotoxicological, hypersensitivity, and inflammatory responses in B6C3F1 mice.

Accomplishing this first objective requires several tasks to be completed:

1. Completion of range finding studies to determine the No Observed and Low Observed Immunological Effect Level (NOIEL and LOIEL, respectively) after a 14-day exposure to the single agents DEET, PYR, or JP-8 in female B6C3F1 mice (Experiments #1A-C of original proposal).
2. Assessing immunological effects of concurrent exposure to JP8, DEET, and PYR at the following levels: 1/3 the NOIEL level, the NOIEL, and the LOIEL (Experiments #1D-G of original proposal) on general immune parameters, antibody

production to a T-cell dependent antigen, the delayed-type hypersensitivity response, and on cytotoxic T-cell activity.

According to the original time table the project is on schedule (Figure 1). Range finding studies have been completed and the NOIEL and LOIEL have been identified for each of the agents. In addition, studies assessing immune parameters following concurrent exposure to DEET, PYR, and JP-8 (also referred to as 'mixture' study) have been initiated.

To improve the assessment of the mixture study, we have modified the experimental design slightly. We originally proposed to conduct experiments consisting of one control group and four treatment groups (1/3 NOIEL of PYR, DEET, & JP-8, NOIEL of PYR, DEET, & JP-8, LOIEL of PYR, DEET, & JP-8) (Figure 2). However, we currently propose to conduct these experiments using one control group and five treatment groups (PYR only, DEET only, JP-8 only, and the combined exposure group) (Figure 3). Therefore, in the first experiment the 1/3 NOIEL would be assessed, in the second the NOIEL would be assessed, and in the third experiment the LOIEL would be assessed. As this modification will require slightly more effort, we believe that this approach will allow for a more direct comparison of single versus concurrent exposures. Furthermore, many of the immunological assays exhibit a seasonal effect and it is important to assess both single and concurrent exposures at the same dose, at the same time of year. This allows for the improved detection of chemical interactions than does relying on historical data from the single exposure range-finding studies. To date the concurrent exposure studies at the LOIEL level have been assessed twice and a third experiment will be completed on February 29, 2000. The studies with the NOIEL and the 1/3 NOIEL will be completed within the next two months.

3. Assessment of serum IgM and IgG levels, Delayed Type Hypersensitivity, and Cytotoxic T-cell activity following concurrent exposure to DEET, PYR, and JP-8. Experiments (#1E-G as in original proposal) are scheduled for year two of the project and are required to complete the first specific aim.

These experiments are scheduled to begin in May 2000.

Year 3- Specific Aim (Objective) 2: To test the hypothesis that 14-day concurrent exposure to DEET, PYR, and JP-8 will increase susceptibility to infectious disease or cancer in female B6C3F1 mice. As this objective will be completed the third year of the project, this work has not begun.

Year 3- Specific Aim (Objective) 3: To test the hypothesis that 14-day concurrent exposure to DEET, PYR, and JP-8 will accelerate autoimmune disease in autoimmune prone mice. As this objective will be completed the third year of the project, this work has not begun.

PRELIMINARY RESULTS AND DISCUSSION- YEAR 1

Note: In the range finding studies the assessment of total serum IgM and IgG levels was replaced with the plaque-forming cell assay, because it is a more functional determination of B-cell activity. In addition, macrophage assessment (phagocytosis, nitrite production, and tumor necrosis factor-alpha production) after single and concurrent exposure to DEET, PYR, and JP-8 will be evaluated in year 2 rather than year 1 of the study.

Range Finding Studies

JP-8 Jet Fuel Single Exposure Studies

Body Weights, Organ Weights, Organ Cellularity, and Peripheral Blood Counts

Treatment with JP-8 only at levels ranging from 500 to 2000 mg/kg/day had no effect on body weight change over the 14-day exposure period (Figure 4). The thymic somatic index {TSI=(thymus wt/body wt)*100} (Figure 5) was decreased following exposure to 2000 mg/kg/day while there was no effect on splenic somatic index {SSI=(spleen wt/body wt)*100} (Figure 6) at the same levels. Alterations were observed in the hepatic somatic index {HSI=(liver wt/bodywt)*100, Figure 7) at levels 1000 and 2000 mg/kg/day. Additionally, treatment with 2000 mg JP-8/kg/day resulted in decreases in thymic cellularity but had no effect on splenic cellularity (Figure 8). No differences in WBC counts or in differential counts were noted in any of the trials (Figures 9,10).

Splenic Lymphocyte Proliferation and NK Cell Function

JP-8 treatment did not cause any alterations in natural killer cell activity at any of the dose levels assessed for any of trials (Figure 11). Furthermore, exposure to JP-8 did not result in any effect on T-or B-cell proliferation in female B6C3F1 mice (Figure 12).

Splenic and Thymic CD4/CD8 Sub-populations

Treatment with JP-8 had no effect on splenic or thymic CD4/CD8 subpopulations (Figures 13-14).

Macrophage Phagocytosis, TNF- α and NO₂ Production

To be completed in year 2.

Antibody Plaque-Forming Cell Assay

Treatment with JP-8 caused significant decreases in the PFC response following treatment with 500, 750, 1000, and 2000 mg/kg/day (Figure 15).

Stomach Histology

Stomachs have been collected, embedded, and sliced. They are scheduled to be analyzed by May 2000.

DEET Single Exposure Studies

Body Weights, Organ Weights, Organ Cellularity, and Peripheral Blood Counts

Mice in all groups appeared to gain weight equally in two of three experiments (Figure 16). However, one range-finding study indicated that mice exposed to DEET gained significantly ($P < 0.0001$) less weight than the control group (Figure 17). Secondary immune organ weights (spleen and thymus) and liver weights exhibited no change as compared to control groups (Figures 18-20). No effect was observed in either peripheral WBC counts or in differential counts (Figures 21-23).

Splenic Lymphocyte Proliferation and Splenic NK Cell Function

Exposure to DEET caused no effect on either T- or B-cell mitogen induced proliferation (Figure 24). Our model indicated that concanavalin A (Con A) was more effective in stimulating T-cell proliferation than was phytohemagglutinin (PHA). Natural killer (NK) cell activity was increased at the 15.5 and 62 mg/kg/day treatment levels but this was not significantly different

($P=0.0839$) from control. The failure to detect significant changes may be due to the experimental variation (standard error) of this experiment (Figure 25). Year 2 studies that will evaluate the single and combined exposures to DEET, PYR, and JP-8 are expected to confirm these preliminary observations.

Splenic and Thymic CD4/CD8 Sub-populations

Subcutaneous treatment with DEET did not affect splenic and thymic CD4/CD8 subpopulations in one experiment. However, inconsistent changes were noted in the second and third trials. In the second trial, treatment with 62 mg/kg/day of DEET resulted in a decrease in the percent of splenic CD4+ cells (Figure 26). Additionally, treatment with 15.5, 31, and 62 mg/kg/day caused a decrease in the percentages of thymic CD4-/CD8- cells (Figure 27). In the third trial, splenic CD4+ cells were again targeted as decreases were noted in the 62 mg/kg/day treatment group (Figure 26-27).

Macrophage Phagocytosis, TNF- α and NO₂ Production

To be completed in year 2.

Antibody Plaque-Forming Cell Assay

Treatment with either 15.5, 31, or 62 mg/kg/day resulted in suppression of the antibody plaque-forming cell response (57%, 53% and 29% of control, respectively; Figure 28). Interestingly, treatment with 7.7 mg/kg/day resulted in a slight stimulation (117% of control) that was not significantly different from control. This observation was not expected; however, this result was remarkably consistent and repeatable.

Pyridostigmine Bromide Single Exposure Studies

Body Weights, Organ Weights, Organ Cellularity, and Peripheral Blood Counts

There was no difference in weight gain in the PYR-treated mice as compared to control mice in any of the trials (Figure 29). No treatment effects were detected in secondary immune organ weights (thymus and spleen) and liver weights (Figures 30-32). Additionally, no effect was observed in either peripheral WBC counts or in differential counts (Figures 33-35).

Splenic Lymphocyte Proliferation and NK Cell Function

Oral exposure to PYR for 14 days did not affect either T- or B-cell mitogen induced proliferation (Figure 36). Natural killer (NK) cell activity was increased at the 10 and 20 mg/kg/day treatment levels but this was not significantly different from controls (Figure 37).

Splenic and Thymic CD4/CD8 Sub-populations

Effects of PYR on CD4/CD8 subpopulations were not consistent between trials. No significant effects were observed in any of the treatment groups as compared to controls in the first trial (Figures 38-39). However, decreases were observed in the percent of thymic and splenic CD4+ cells and thymic CD8+ cells at the 20 mg/kg/day exposure level. Increases in thymic CD4+/CD8+ cells resulted from treatment with 5 or 20 mg/kg/day of PYR (Figures 38-39).

Macrophage Phagocytosis, TNF- α and NO₂ Production

To be completed in year 2.

Antibody Plaque-Forming Cell Assay

The PFC response was suppressed in mice treated with PYR by 62%, 62%, and 33% at the 1, 5 and 20 mg/kg/day exposure levels, respectively (Figure 40). The 10 mg/kg/day level indicated some decrease, however this was not statistically significant (81% of control). Additional experiments indicated that PYR consistently suppressed the PFC response at levels of 2 mg/kg/day levels (43 or 76% of control) and this is consistent with the early trials that presented suppression at 1 and 5 mg/kg/day. This dose response study will be verified this spring in order to clarify the dose-response over the 1-20 m/kg/day range.

JP-8, DEET, & PYR Concurrent Exposure Studies

From the range finding studies it was determined that the LOIEL for the study agents administered for 14 days was: 15.5 mg DEET/kg/day, 500 mg JP-8/kg/day and 1 mg PYR/kg/day. Therefore, with the exception of PYR, these were the levels representative of the LOIEL. The level chosen for PYR was 2 mg/kg/day. This levels is very close to the LOIEL (1 mg/kg/day) and was determined to be more comparable to the level the soldiers were administered.

Concurrent Exposure To JP-8, DEET, and PYR at The Lowest Observable Immune Effect Levels (LOIEL)

Body Weights, Organ Weights, Organ Cellularity, and Peripheral Blood Counts

Treatment with either the LOIEL for each compound or the mixture agents (2 mg/kg/day PYR, 500 mg/kg/day JP-8, or 15.5 mg/kg/day DEET) resulted in no effect on body, spleen, or thymus weights (Figures 41-43). However, increases in the hepatic somatic index [HSI = (liver wt/body wt)*100] were observed in both experimental trials in the mixture treatment (concurrent

exposure group), while the JP-8 treatment was only noted to be different from control in the second trial (Figure 44). Additionally, in both trials the DEET treatment was significantly different from the mixture treatment and in the first trial the PYR treatment was different from the mixture. In neither trial was the JP-8 different from the mixture. These data suggest that the increase in the HSI in the mixture treatment may be due primarily to the JP-8.

Splenic Lymphocyte Proliferation and NK Cell Function

Treatment with either 15.5 mg/kg/day or with the mixture resulted in a significant decrease in the T-cell proliferative response to Con A in the first trial; however, this result was not observed in the second trial (Figure 45). No effect on NK cell activity was observed in any of the treatments in either trial (Figure 46).

Splenic and Thymic CD4/CD8 Sub-populations

No difference in splenic or thymic CD4/CD8 subpopulations were observed following treatment with the LOIELs of the agents (2 mg/kg/day PYR, 500 mg/kg/day JP-8, or 15.5 mg/kg/day DEET) or in the mixture group which received the LOIEL of all the agents (Figures 47-50).

Macrophage Phagocytosis, TNF- α and NO₂ Production

To be completed in year 2.

KEY RESEARCH ACCOMPLISHMENTS

Research accomplishments to date have been primarily focused on meeting specific aim 1 of the study. Baseline effects of the individual agents have been evaluated and the mixture

studies have been initiated. In the case of the NK assay and the flow cytometric evaluation of lymphocytic subpopulations, variability in the results has precluded a concise effect of the agents. This is easily remedied as the modified experimental design will permit repeat evaluations of the single agents and the mixture in the same experiment. This approach, although it requires more effort, is expected to elucidate interactive effects of the agents that is fundamental to this study.

REPORTABLE OUTCOMES

Manuscripts, Abstracts and Presentations

Manuscripts:

None in calendar year 1999

Presentations:

None in calendar year 1999.

Abstracts:

Three abstracts have been submitted and accepted by the Society of Toxicology for the national meeting in Philadelphia, PA, **March 2000**.

Gilkeson, G.S., Dudley, A.C., EuDaly, J., Peden-Adams, M.M., and Keil, D.E.
Effects of N,N-diethyl-m-toluamide (DEET) on immune function parameters in
B6C3F1 mice. Accepted to The Toxicologist, 2000.

Preliminary Data- Do not Cite or Quote Without the Author's Permission

Peden-Adams, M.M., Dudley, A.C., EuDaly, J., Gilkeson, G.S., and Keil, D.E. Effects of exercise stress or pyridostigmine bromide (PSB) on immune function parameters in B6C3F1 mice. Accepted to The Toxicologist, 2000.

Dudley, A.C., EuDaly, J., Peden-Adams, M.M., and Keil, D.E. An aryl hydrocarbon receptor independent mechanism of JP-8 jet fuel immunotoxicity in two strains of mice. Accepted to The Toxicologist, 2000.

Patents and Licenses Applied for and/or Issued

None in calendar year 1999.

Degrees Obtained that are supported by this award

Andrew Dudley, B.S., is a master's level student in the Environmental Science Program at MUSC. He is actively participating by conducting range-finding studies and multi-exposure experiments. He is assessing a variety of immunological endpoints to include natural killer activity, lymphocyte proliferative function, and the quantification of lymphocyte subpopulations in immune organs. In addition, his work has explored potential mechanisms for JP-8 related immunosuppression and this information is pertinent to this study. He presented some of his research in January 2000 at the AFOSR JP-8 Workshop held in Tucson, AZ. He is expected to complete his portion of the study by graduation of Summer, 2000. In addition, he has been accepted to the University of Melbourne in Australia where he plans to complete a doctoral research degree.

Kimberlee Banks, B.S. is a master's level student in the Medical Laboratory Sciences Program at MUSC. She is also participating by determining the effect of the concurrent

exposure to DEET, PYR, and JP-8 on antibody production. She is expected to complete her research portion and meet graduation requirements by May, 2000.

Development of cell lines, tissue or serum repositories

None in calendar year 1999.

Informatics such as Databases and animal models

None in calendar year 1999.

Funding applied for based on this work

None in calendar year 1999.

Employment or research opportunities applied for and/or received on experiences/training supported by this award

None in calendar year 1999.

CONCLUSIONS

This report contains data collected from the first year of a three year study. As this is early in the progression of this project, identification of any detrimental immunological effects due to the concurrent exposure of DEET, PYR, and JP-8 is limited and can not be reported at this time. However, we do report data regarding immunosuppression from exposure to the individual agents.

Information regarding the immunotoxicological profile of DEET, PYR and more recently, JP-8, have been limited to non-existent. Few reports have been recently published regarding the immunotoxic consequences caused by JP-8 via various routes of exposure. Studies

performed by Drs. David Harris et al., (12) and Steve Ullrich (13) indicate that JP-8 via inhalation or dermal exposure targets the thymus, spleen, liver, and the functional endpoint, T cell proliferation. Our study utilizes an oral exposure to JP-8. This has provided interesting information regarding JP-8 exposure as the immune effects due to oral JP-8 are slightly different than when it is administered via the aerosolized or dermal route. As we have identified similar changes in the liver and thymus as reported in the dermal and inhalation studies, these changes have not been as pronounced. In addition, our preliminary study suggests that the spleen and T cell proliferation endpoints are not significantly affected when JP-8 is administered orally. However, oral JP-8 at low exposure levels (500 mg/kg/day for 14 days) targets B cell function and decreases their ability to produce IgM antibody to specific antigens. Furthermore, this same effect on B cells has been observed in mice administered low levels of PYR (1, 2, and 5 mg/kg/day for 14 days) or DEET (15.5 mg/kg/day for 14 days).

The preliminary studies indicate that DEET, PYR, or JP-8 individually cause deleterious effects to the immune system. Although not all immune endpoints have been affected, these agents seem to primarily and consistently suppress B cell function according to our existing data.

In addition to assessing the immunotoxic effects of JP-8, studies in our laboratory are exploring potential mechanism(s) by which JP-8 mediates its immunotoxicity. Data collected from this is expected to be invaluable for understanding potential interaction effects from concurrent exposure to DEET, PYR, and JP-8.

JP-8 jet fuel is a complex mixture of aliphatic and aromatic hydrocarbons. Several of the compounds found in this mixture are known to preferentially activate various enzyme classes of the cytochrome P450 (CYP450) family. For example, benzene affects CYP4502E1 while naphthalene alters the function of CYP4501A1. The route of exposure is important in determining the effects of compounds such as JP-8, whose toxicity may be mediated through the CYP450 system. Oral exposures may have either a greater or lesser physiological effect than other routes of exposure since compounds introduced in this way will affect the liver before entering the

systemic circulation. Thus, JP-8 may be detoxified or bioactivated before circulating through the body. In light of other recent studies, current results suggest that JP-8 may be detoxified via phase I metabolism (CYP450) and/or phase II metabolism pathways. Glutathione-s-transferase, a phase II enzyme, has been shown to be induced by exposure to JP-8 (unpublished data). Furthermore, the observed increases in liver weights are often indicative of CYP450 induction. Preliminary assessment of western blotting to determine induction of CYP4501A1/2 and Ah-Receptor (AhR) binding following JP-8 exposure, however, indicates that JP-8 does not bind to the Ah-R or induce CYP4501A1/2 (Dudley et al., 2000). Although an increase in protein synthesis was not observed, cytochrome P450 enzyme activity could be increased potentially allowing for greater detoxification. To determine this, EROD and MROD enzymatic endpoints should be assessed. Moreover, oral JP-8 exposure may not be as good of a "worst case" scenario model as might have been expected. To accurately model JP-8 toxicity, differences in the effects of exposure routes should be assessed with all other parameters (i.e.: dose, frequency, and duration) held constant and determination of the effect of JP-8 on both Phase I and Phase II metabolism should be evaluated.

REFERENCES

1. Amato AA, McVey A, Cha C, Matthews ED, Jackson CE, Kleingunther R, Worley L, Cornman E, and Kagan-Hallet K. 1997. Evaluation of neuromuscular symptoms in veterans of the Persian Gulf War. *Neurol.* 48(1):4-12.
2. Wittich AC. 1996. Gynecologic evaluation of the first female soldiers enrolled in the Gulf War Comprehensive Clinical Evaluation Program at Tripler Army Medical Center. *Military Med.* 161(11):635-637.
3. Haley RW, and Kurt TL. 1997. Self-reported exposure to neurotoxic chemical combinations in the gulf war. *JAMA.* 277(3):231-7.
4. Gouge SF, Daniels DJ, and Smith CE. Exacerbation of asthma after pyridostigmine during operation desert storm. *Military Med.* 159(2):108-11.

5. Retrospective analysis of calls to poison control centers resulting from exposure to the insect repellent *N,N*-Diethyl-*m*-toluamide (DEET) from 1985-1989. *Clin. Toxicol.* 32(1):1-16.
6. Osmitz TB and Grothaus RH. 1995. The present safety assessment of deet. *J. Amer. Mosquito Control Assoc.* 11:274-278.
7. Wenger CB, and Latzka WA. 1992. Effects of pyridostigmine bromide on physiological responses to heat, exercise, and hypohydration. *Aviat. Space Environ. Med.* 63(1):37-45.
8. Cook JE, Kolka MA, and Wenger CB. 1992. Chronic pyridostigmine bromide administration: side effects among soldiers working in a desert environment. *Military Med.* 157(5):250-4.
9. Friedman A, Kaufer D, Shemer J, Hendler I, Soreq H, and Tur-Kaspa I. 1996. Pyridostigmine brain penetration under stress enhances neuronal excitability and induces early immediate transcriptional response. *Nat. Med.* 2(12):1382-5.
10. Abou-Donia MB, Wilmarth KR, Jensen KF, Oehme FW, and Kurt TL. 1996. Neurotoxicity resulting from coexposure to pyridostigmine bromide, deet, and permethrin: implications of Gulf War chemical exposures. *J. Toxicol. Environ. Health.* 48(1)35-56.
11. Abou-Donia MB, Wilmarth KR, Abdel-Rahman AA, Jensen KF, Oehme FW, and Kurt TL. 1996. Increased neurotoxicity following concurrent exposure to pyridostigmine bromide, DEET, and chlorpyrifos. *Fundam. Appl. Toxicol.* 34(2):201-22.
12. Harris DT, Sakiestewa D, Robledo RF, and Witten M. 1997. Immunotoxicological effects of JP-8 jet fuel exposure. *Toxicol. Indust. Health.* 13(1):43-55.
13. Ullrich, S. E. 1999. Dermal application of JP-8 jet fuel induces immune suppression. *Toxicol. Sciences* 52:61-67

APPENDIX A
Figures and Graphs

<p>Experiment 1</p> <p>LOIEL-Single chemical and concurrent exposure.</p>			<p>Experiment 2</p> <p>LOIEL-Single chemical and concurrent exposure.</p>		
Treatment 1:			Treatment 1:		
Control			Control		
Treatment 2:			Treatment 2:		
LOIEL of JP-8			NOIEL of JP-8		
Treatment 3:			Treatment 3:		
LOIEL of PYR			NOIEL of PYR		
Treatment 4:			Treatment 4:		
LOIEL of DEET			NOIEL of DEET		
Treatment 5:			Treatment 5:		
LOIEL JP8 + LOIEL of PYR + LOIEL of DEET			NOIEL JP8 + NOIEL of PYR + NOIEL of DEET		

19

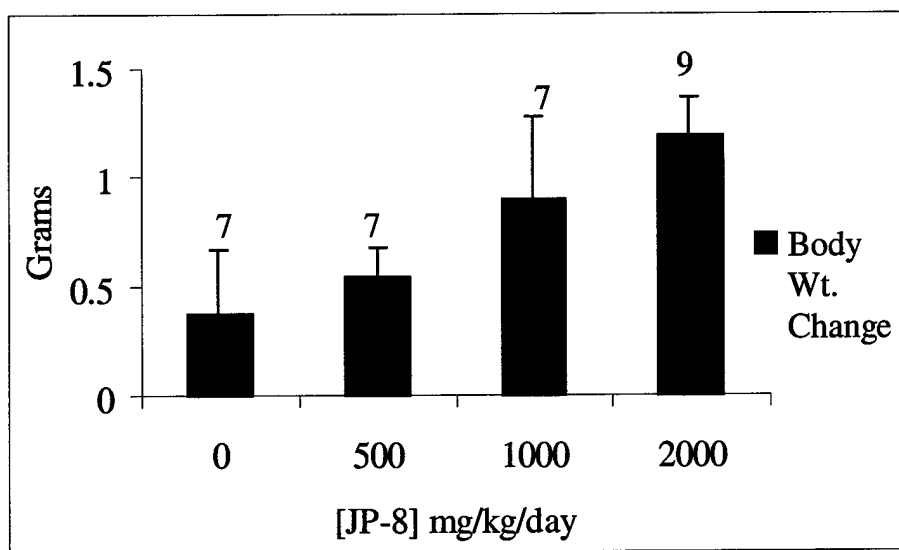


Figure 4. Body weight change in female B6C3F1 mice following a 14-day oral exposure to JP-8 jet fuel. Data is representative of three trials and is presented as mean \pm SEM. Numbers above SEM bars indicate the sample size. $\alpha=0.05$.

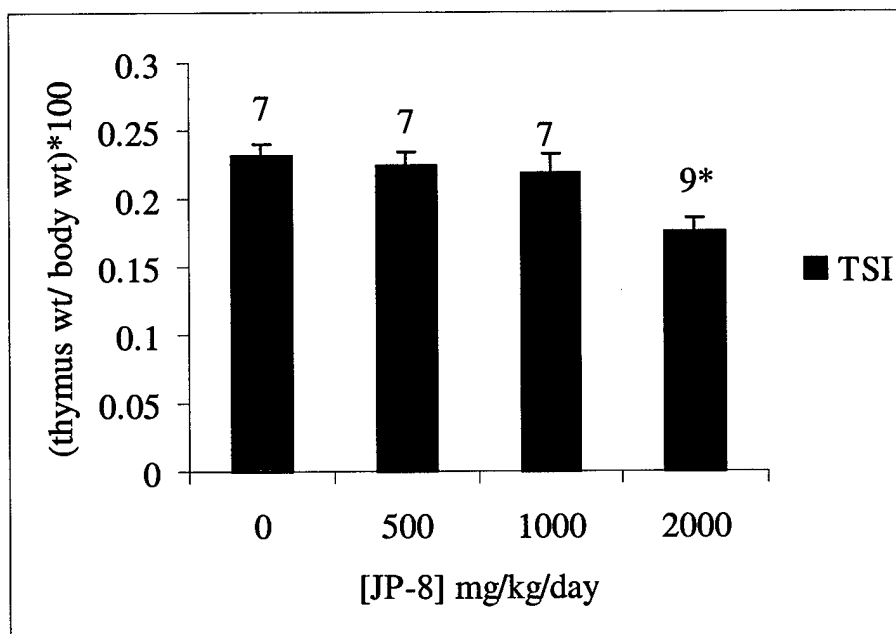


Figure 5. Thymic somatic index {TSI=(thymus wt/body wt)*100} in female B6C3F1 mice following a 14-day oral exposure to JP-8 jet fuel. Data is representative of three trials and is presented as mean \pm SEM. Numbers above SEM bars indicate the sample size. *Significantly different from control. $\alpha=0.05$.

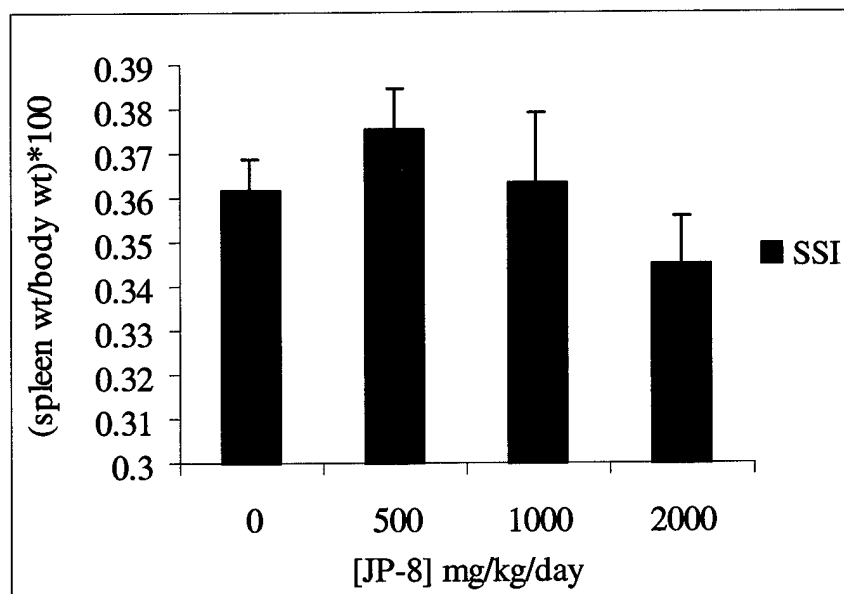


Figure 6. Splenic somatic index {SSI=(spleen wt/body wt)*100} in female B6C3F1 mice following a 14-day oral exposure to JP-8 jet fuel. Data is representative of three trials and is presented as mean \pm SEM. Numbers above SEM bars indicate the sample size. $\alpha=0.05$.

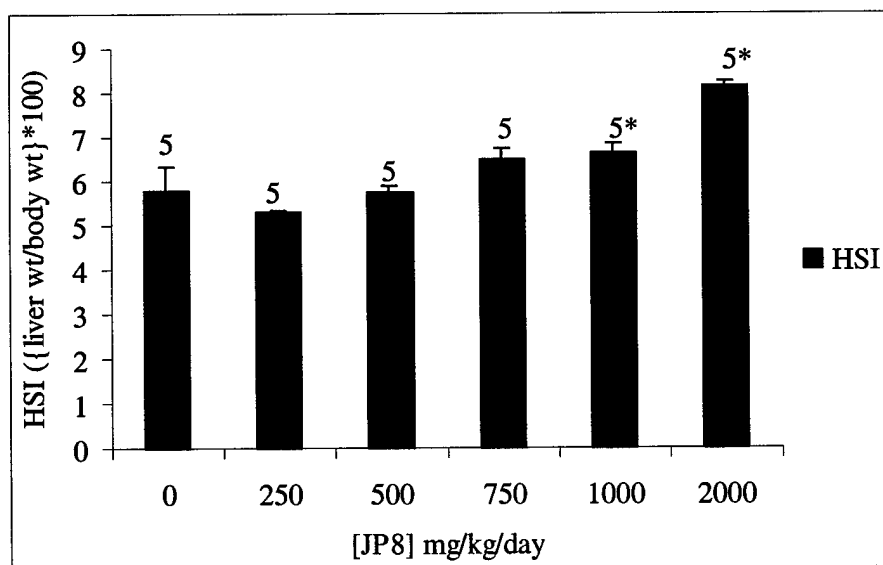


Figure 7. Hepatic somatic index {HSI=(liver wt/body wt)*100} in female B6C3F1 mice following a 14-day oral exposure to JP-8 jet fuel. Data is representative of three trials and is presented as mean \pm SEM. Numbers above SEM bars indicate the sample size. *Significantly different from control. $\alpha=0.05$.

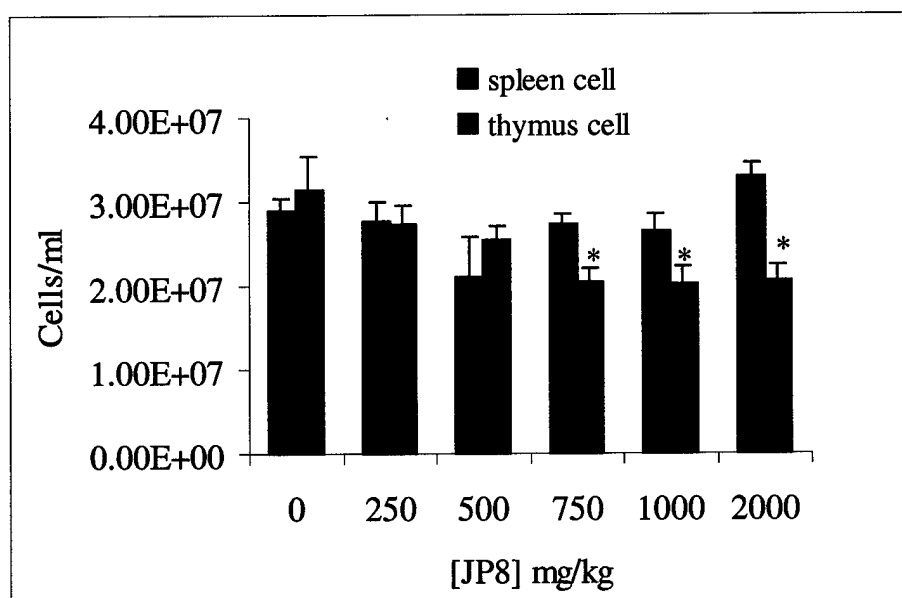


Figure 8. Splenic and thymic cellularity in female B6C3F1 mice following a 14-day oral exposure to JP-8 jet fuel. Data is representative of three trials and is presented as mean \pm SEM. Sample sizes for all groups are 5. *Significantly different from control. $\alpha=0.05$.

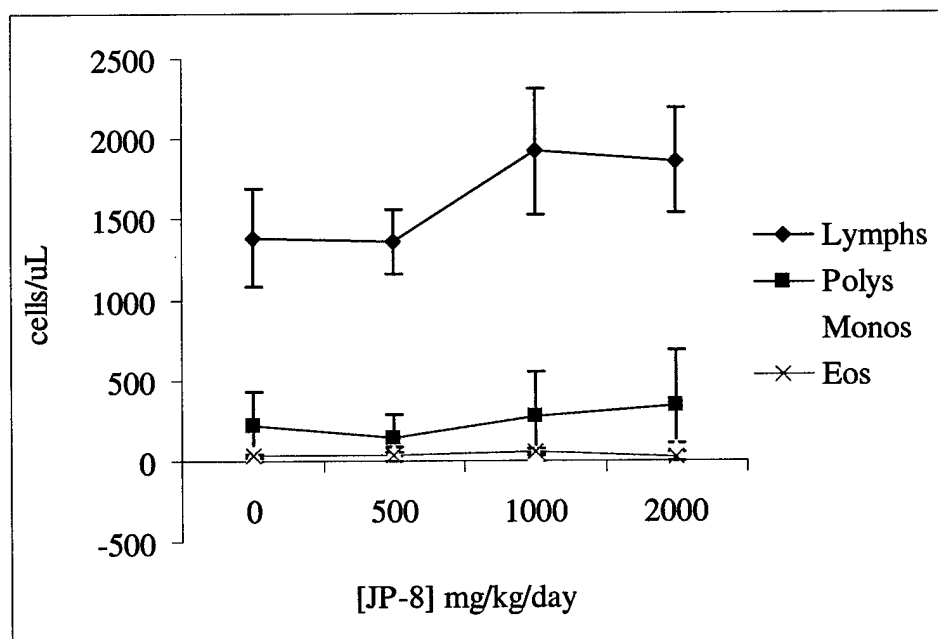


Figure 9. Peripheral blood differential counts in female B6C3F1 mice following a 14-day oral exposure to JP-8 jet fuel. Data is representative of three trials and is presented as mean \pm SEM. Sample size for 0-1000mg/kg/day treatment is 5, and is 9 for the 2000mg/kg/day treatment. $\alpha=0.05$.

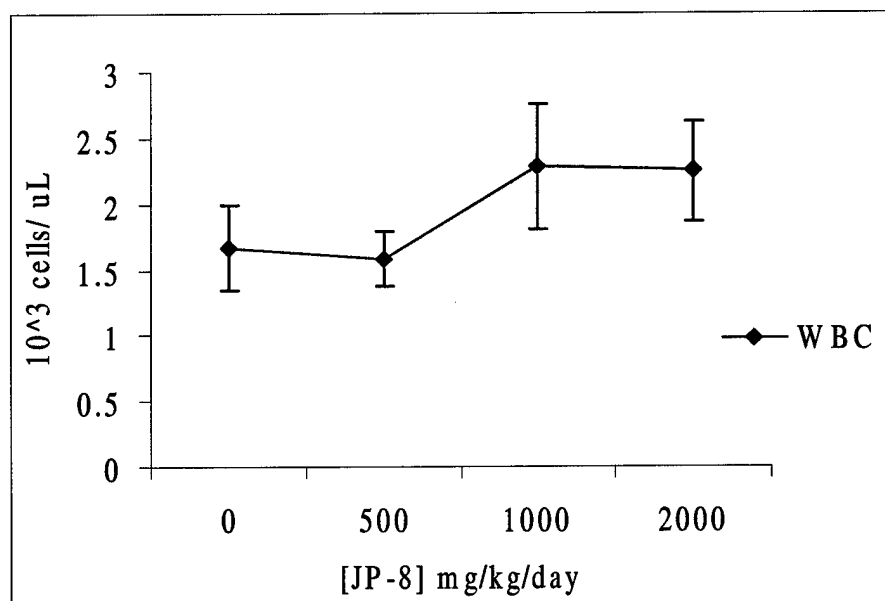


Figure 10. Peripheral white blood cell (WBC) counts in female B6C3F1 mice following a 14-day oral exposure to JP-8 jet fuel. Data is representative of three trials and is presented as mean \pm SEM. Sample size for 0-1000mg/kg/day treatment is 5, and is 9 for the 2000mg/kg/day treatment. $\alpha=0.05$.

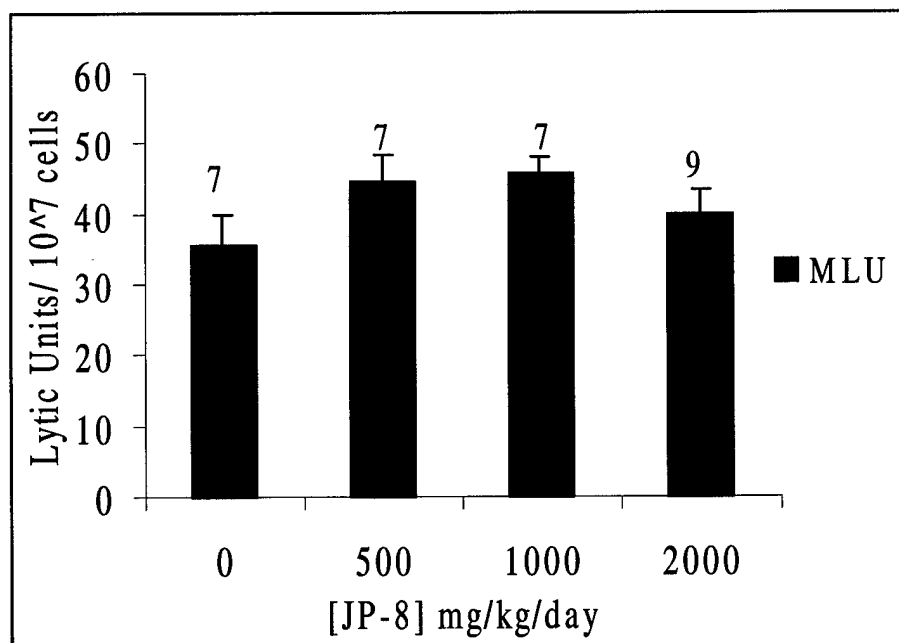


Figure 11. Natural killer cell activity in female B6C3F1 mice following a 14-day oral exposure to JP-8 jet fuel. Con A and PHA are T-cell mitogens and LPS is a B-cell mitogen. Data is representative of three trials and is presented as mean \pm SEM. Sample size for all treatments is 5. $\alpha=0.05$.

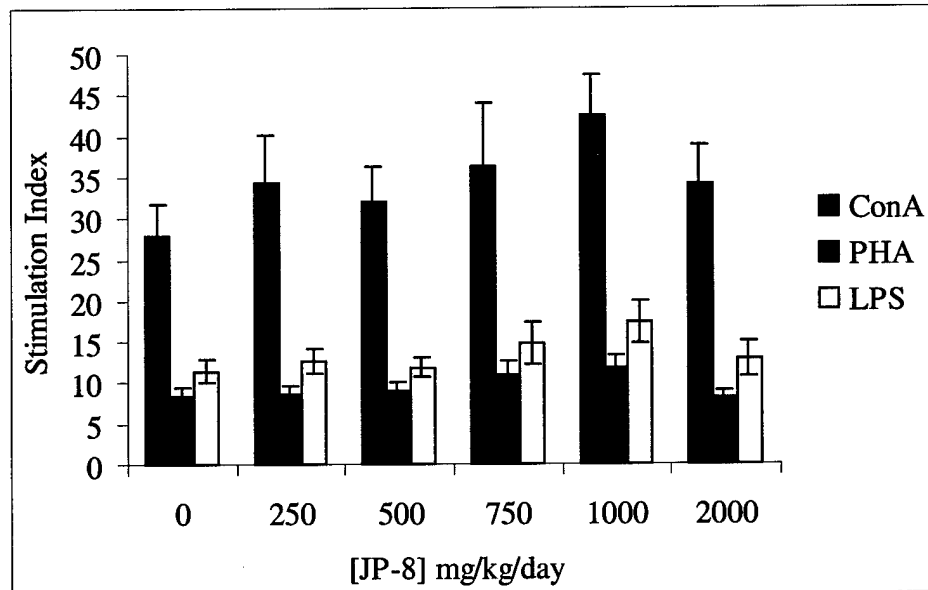


Figure 12. T- and B-cell proliferation in female B6C3F1 mice following a 14-day oral exposure to JP-8 jet fuel. Con A and PHA are T-cell mitogens and LPS is a B-cell mitogen. Data is representative of three trials and is presented as mean \pm SEM. Sample size for all treatments is 5. $\alpha=0.05$.

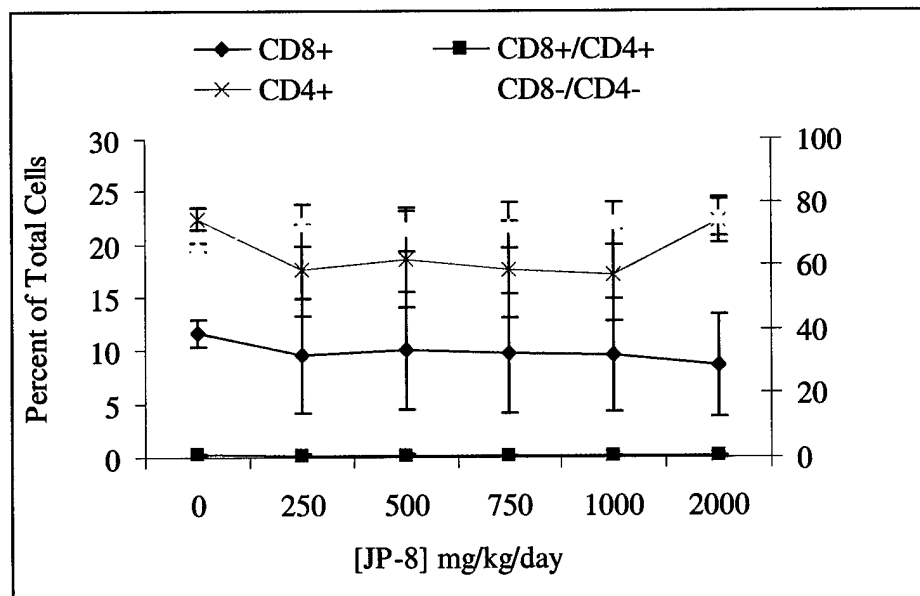


Figure 13. Splenic CD4/D8 subpopulations in female B6C3F1 mice following a 14-day oral exposure to JP-8 jet fuel. Data is representative of three trials and is presented as mean \pm SEM. Sample size for all treatments is 5. Percent CD4-/CD8- is on the secondary axis. $\alpha=0.05$.

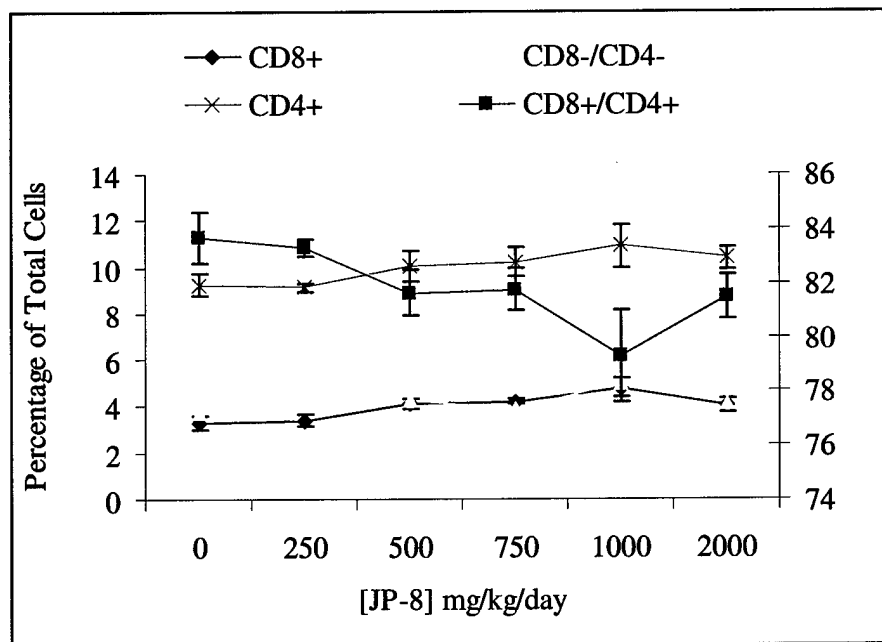


Figure 14. Thymic CD4/CD8 subpopulations in female B6C3F1 mice following a 14-day oral exposure to JP-8 jet fuel. Data is representative of three trials and is presented as mean \pm SEM. Sample size for all treatments is 5. $\alpha=0.05$.

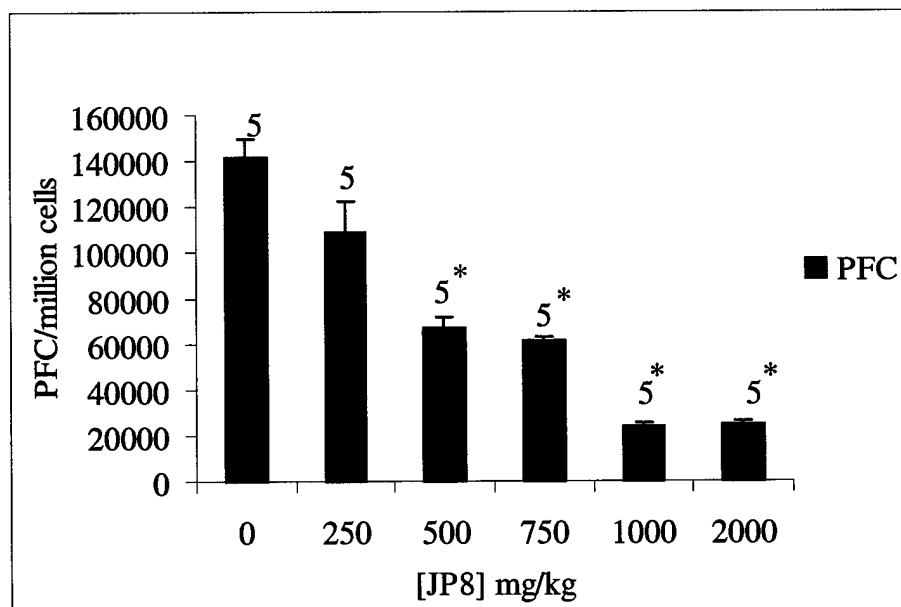


Figure 15. Antibody plaque forming-cell response in female B6C3F1 mice following a 14-day oral exposure to JP-8 jet fuel. Data is representative of three trials and is presented as mean \pm SEM. Sample size for all treatments is 5. *Significantly different from control. $\alpha=0.05$.

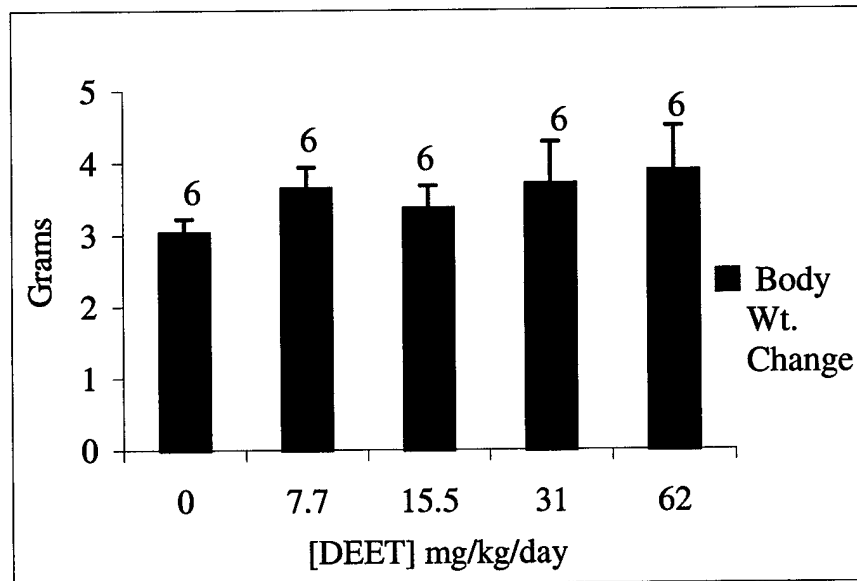


Figure 16. Body weight change in female B6C3F1 mice following a 14-day subcutaneous exposure to N,N-diethyl-m-toluamide (DEET). Data is representative of the range finding trials 2 and 3. Data is presented as mean \pm SEM. Numbers above SEM bars indicate the sample size. $\alpha=0.05$.

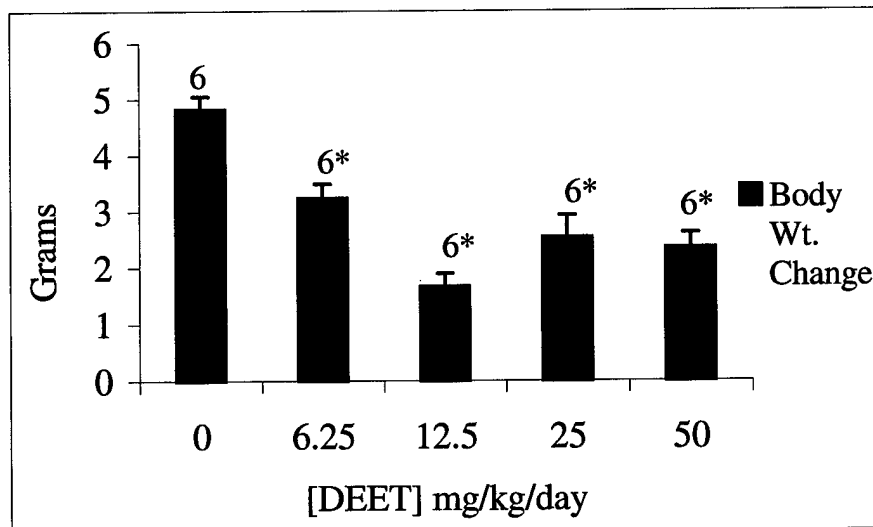


Figure 17. Body weight change in female B6C3F1 mice following a 14-day subcutaneous exposure to N,N-diethyl-m-toluamide (DEET). Data is from the first range finding trial and is presented as mean \pm SEM. Numbers above SEM bars indicate the sample size. *Significantly different from control. $\alpha=0.05$.

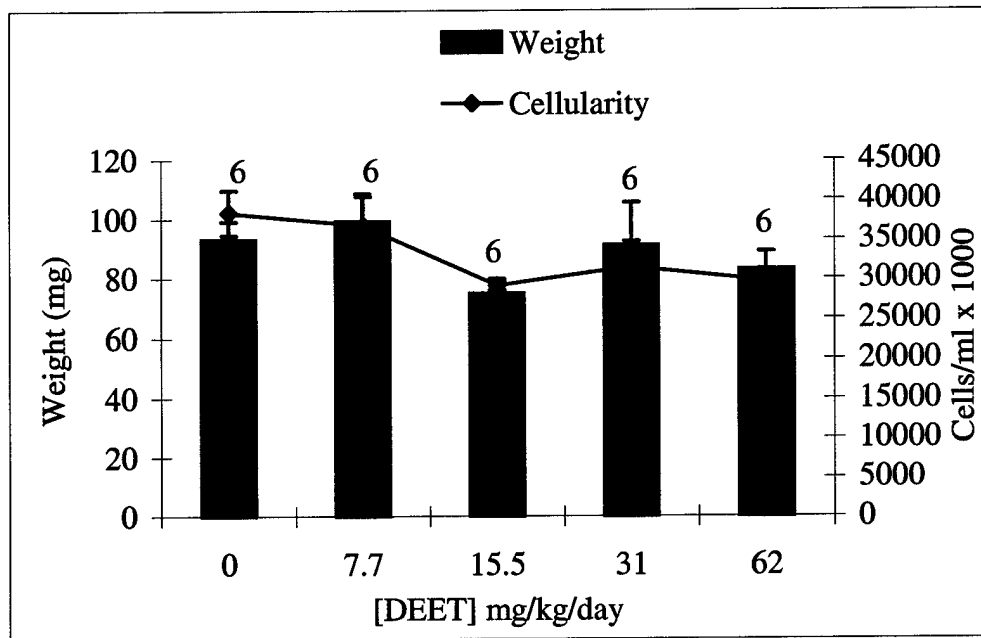


Figure 18. Spleen weights and cellularity in female B6C3F1 mice following a 14-day subcutaneous exposure to N,N-diethyl-m-toluamide (DEET). Data is representative of three trials and is represented as mean \pm SEM. Numbers above SEM bars indicate the sample size. $\alpha=0.05$

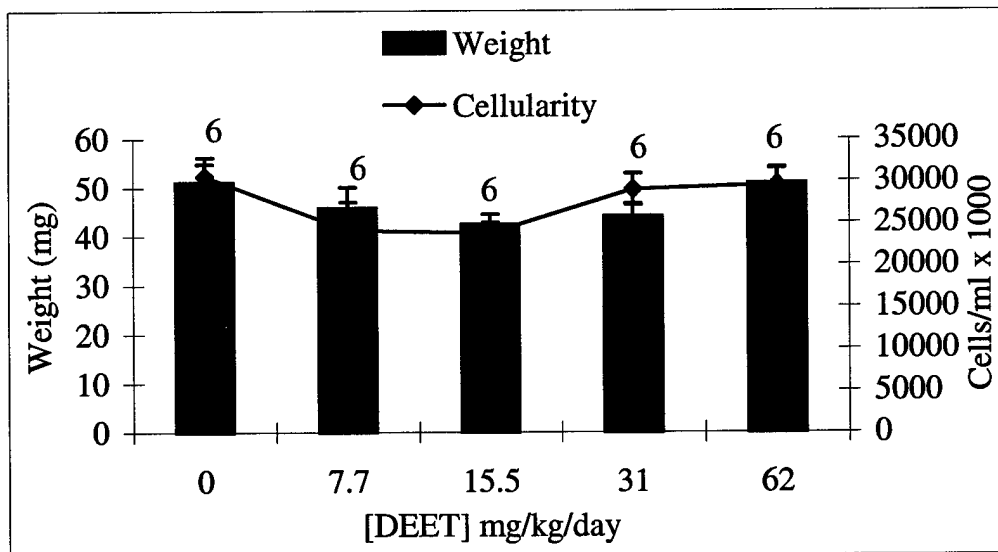


Figure 19. Thymus weights and cellularity in female B6C3F1 mice following a 14-day subcutaneous exposure to N,N-diethyl-m-toluamide (DEET). Data is representative of three trials and is represented as mean \pm SEM. Numbers above SEM bars indicate the sample size. $\alpha=0.05$

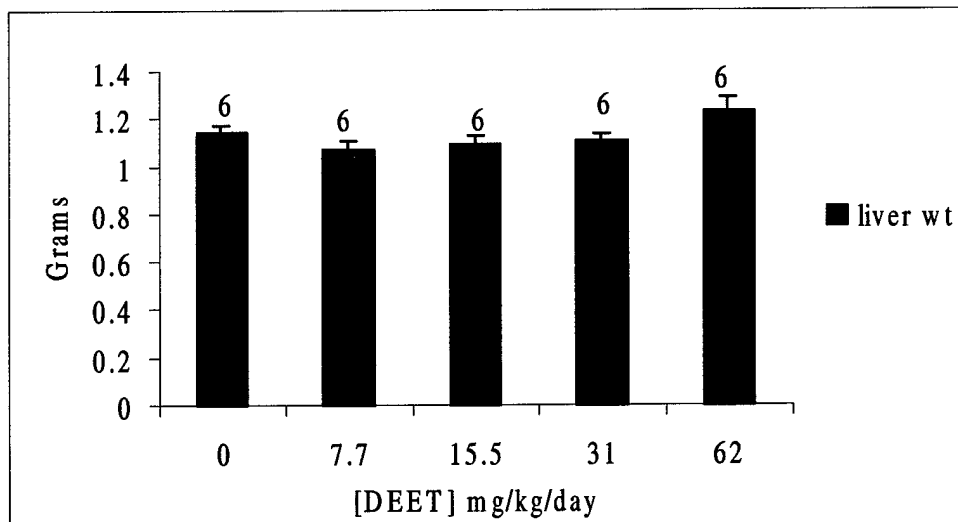


Figure 20. Liver weights in female B6C3F1 mice following a 14-day subcutaneous exposure to N,N-diethyl-m-toluamide (DEET). Data is representative of three trials and is represented as mean \pm SEM. Numbers above SEM bars indicate the sample size. $\alpha=0.05$

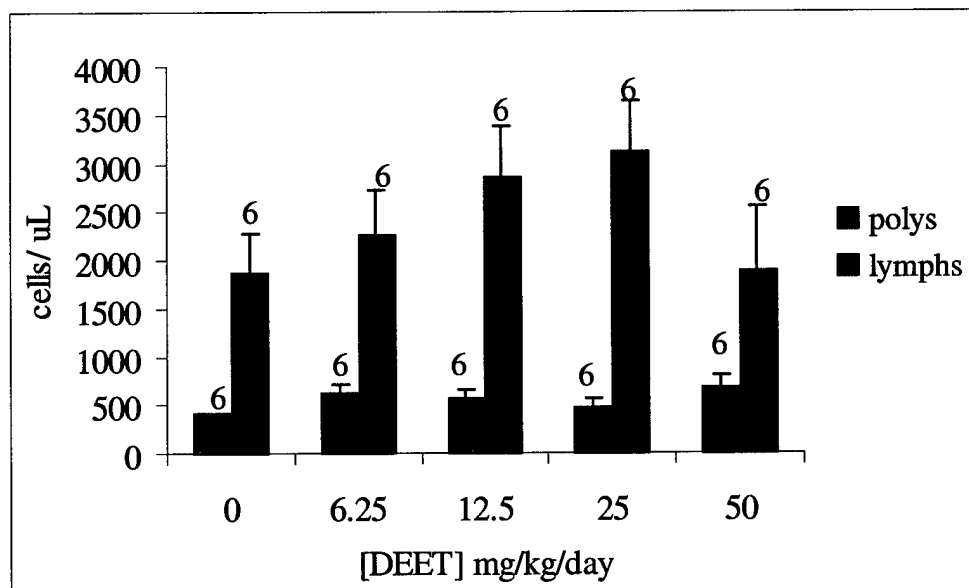


Figure 21. Peripheral blood lymphocyte and PMN counts in female B6C3F1 mice following a 14-day subcutaneous exposure to N,N-diethyl-m-toluamide (DEET). Data is representative of three trials and is represented as mean \pm SEM. Numbers above SEM bars indicate the sample size. $\alpha=0.05$

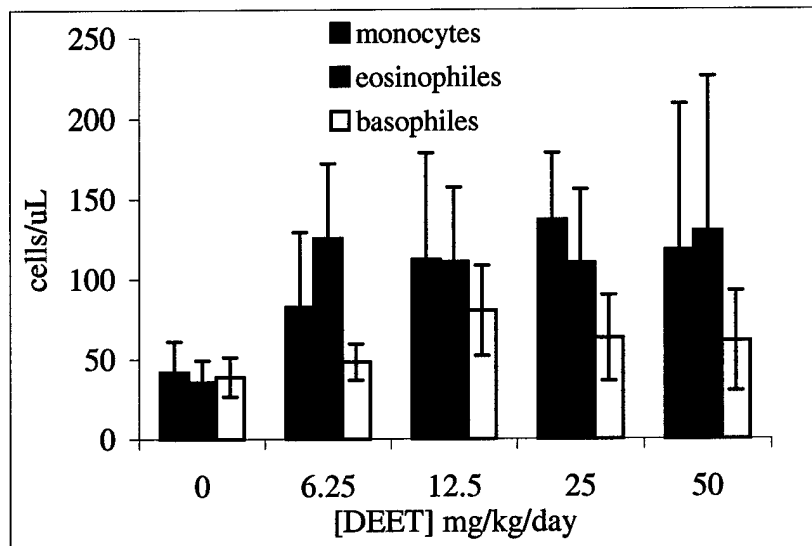


Figure 22. Peripheral blood monocyte, eosinophil, and basophil counts in female B6C3F1 mice following a 14-day subcutaneous exposure to N,N-diethyl-m-toluamide (DEET). Data is representative of three trials and is represented as mean \pm SEM. The sample size for all treatments is 6. $\alpha=0.05$

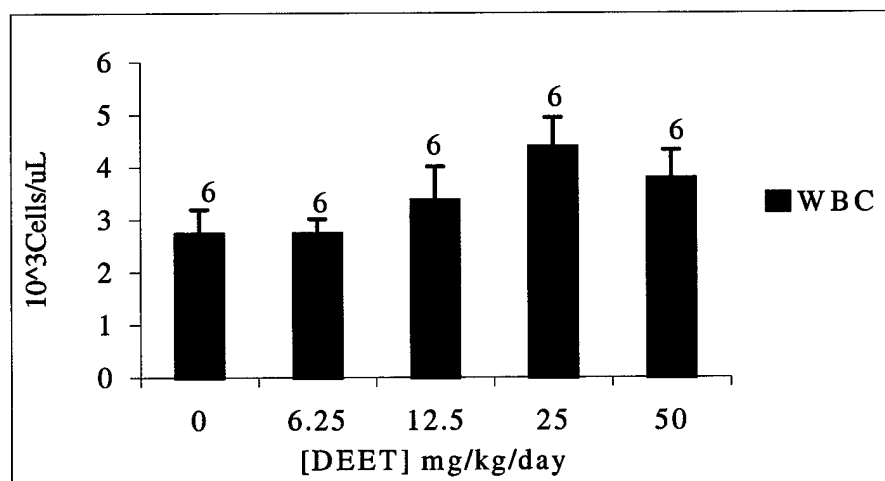


Figure 23. Peripheral blood white blood cell counts in female B6C3F1 mice following a 14-day subcutaneous exposure to N,N-diethyl-m-toluamide (DEET). Data is representative of three trials and is represented as mean \pm SEM. Numbers above SEM bars indicate the sample size. $\alpha=0.05$

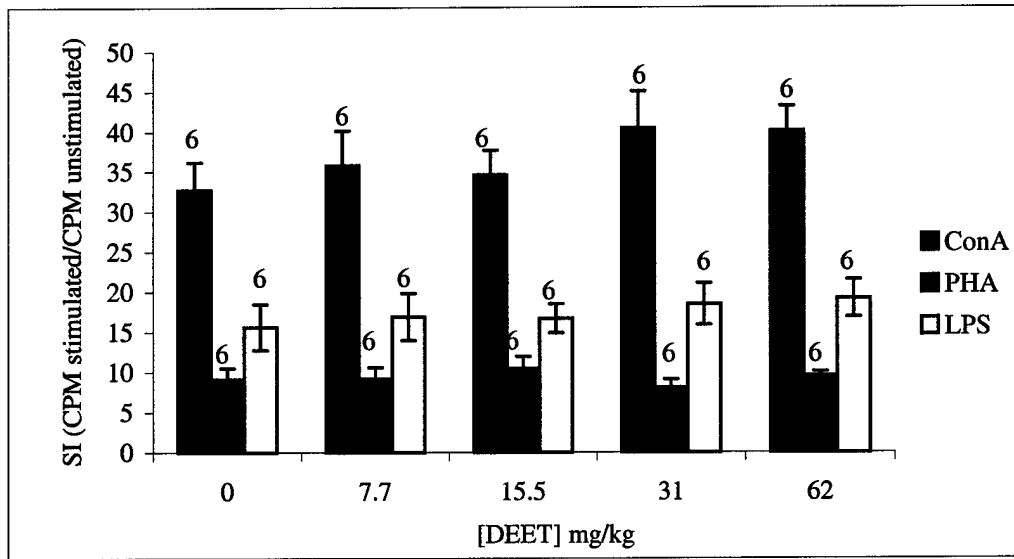


Figure 24. T- and B-cell proliferation in female B6C3F1 mice following a 14-day subcutaneous exposure to N,N-diethyl-m-toluamide (DEET). Con A and PHA are T-cell mitogens and LPS is a B-cell mitogen. Data is representative of three trials and is represented as mean \pm SEM. Numbers above SEM bars indicate the sample size. $\alpha=0.05$

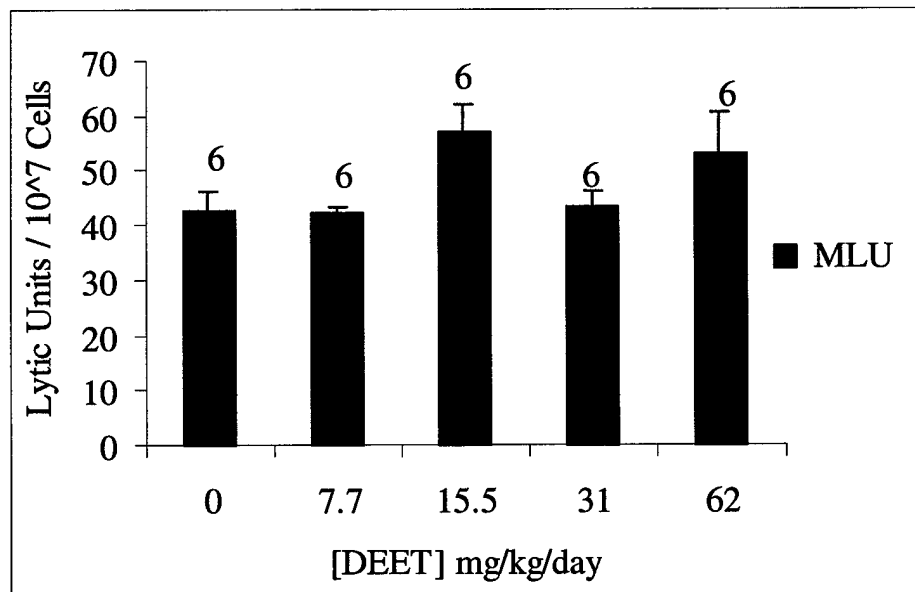


Figure 25. Natural killer cell activity in female B6C3F1 mice following a 14-day subcutaneous exposure to N,N-diethyl-m-toluamide (DEET). Data is representative of three trials and is represented as mean \pm SEM. Numbers above SEM bars indicate the sample size. $\alpha=0.05$

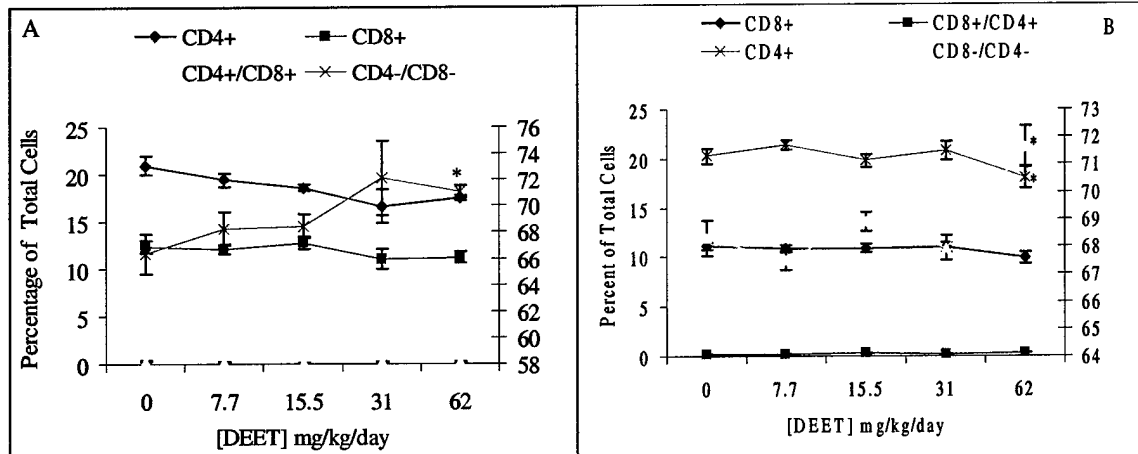


Figure 26. Splenic CD4/CD8 subpopulations in female B6C3F1 mice following a 14-day subcutaneous exposure to N,N-diethyl-m-toluamide (DEET). Data presented as mean \pm SEM. Sample size for all treatments in both trials is 6. $\alpha=0.05$. A= Trial 2, % CD4/CD8- is on secondary axis; B= Trial 3, % CD4/CD8- is on secondary axis.

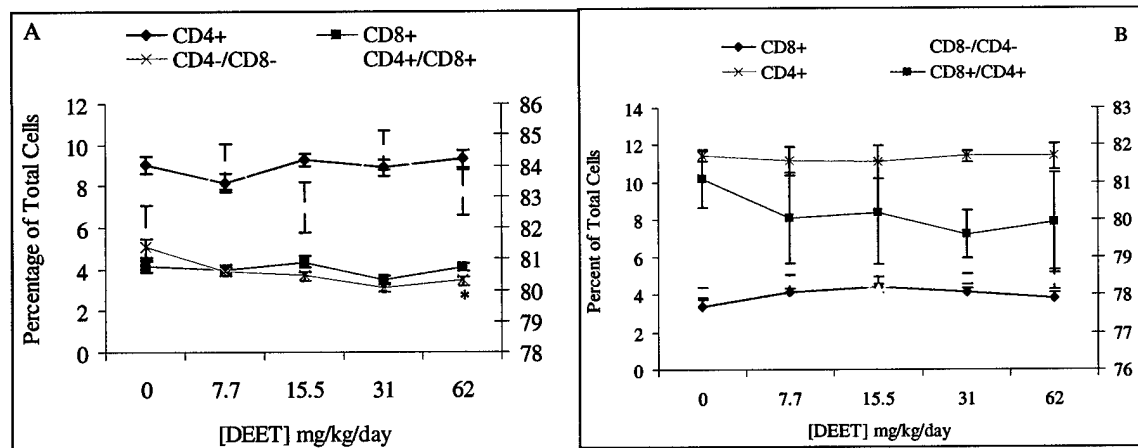


Figure 27. Thymic CD4/CD8 subpopulations in female B6C3F1 mice following a 14-day subcutaneous exposure to N,N-diethyl-m-toluamide (DEET). Data is presented as mean \pm SEM. Sample size for all treatments in both trials is 6. $\alpha=0.05$. A= Trial 2, % CD4+/CD8+ is on secondary axis; B= Trial 3, % CD4+/CD8+ is on secondary axis.

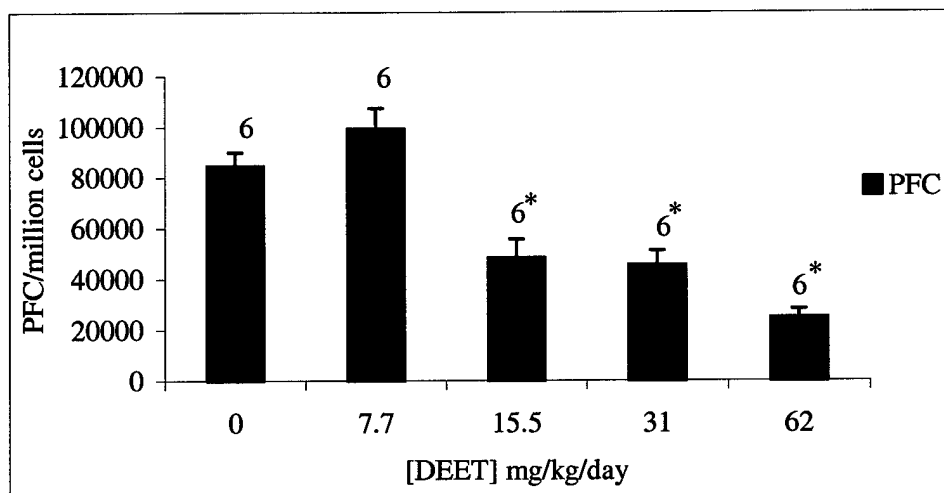


Figure 28. Plaque-forming cell activity in female B6C3F1 mice following a 14-day subcutaneous exposure to N,N-diethyl-m-toluamide (DEET). Data is representative of three trials and is represented as mean \pm SEM. Numbers above SEM bars indicate the sample size. $\alpha=0.05$.

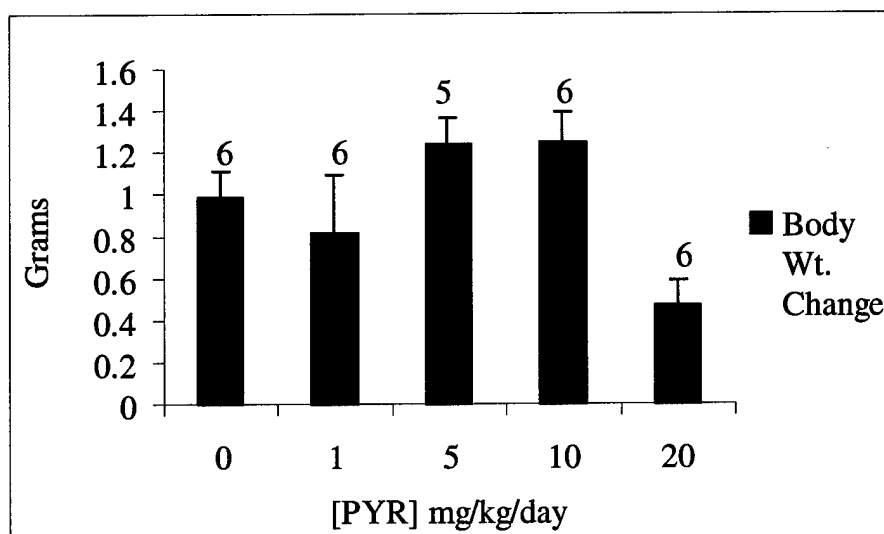


Figure 29. Body weight change in female B6C3F1 mice following a 14-day oral exposure to Pyridostigmine (PYR). Data is representative of two trials and is presented as mean \pm SEM. Numbers above SEM bars indicate the sample size. There are only 5 animals in the 5 mg/kg/day treatment because one died early due to a cranial concussion. $\alpha=0.05$.

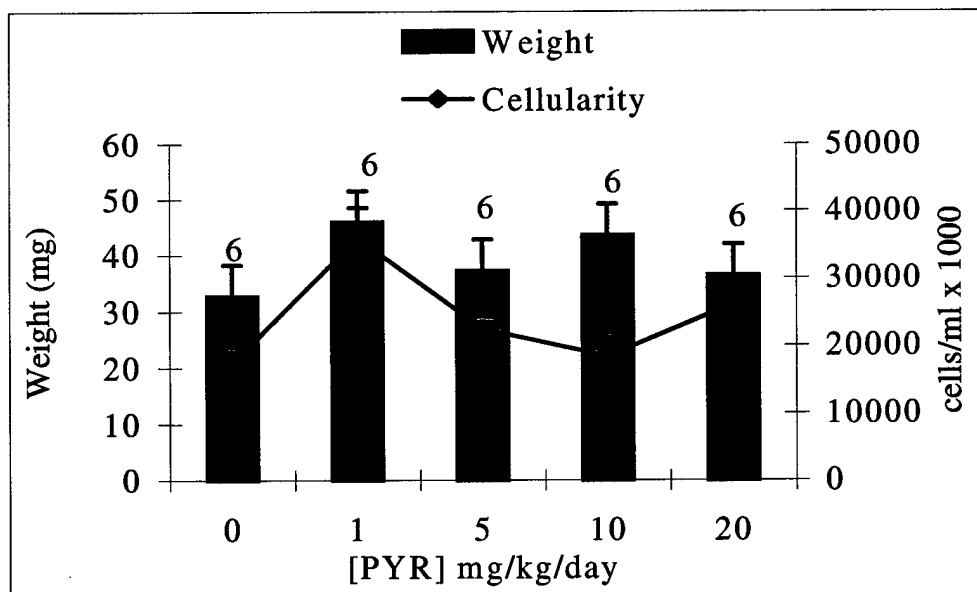


Figure 30. Thymus weights and cellularity in female B6C3F1 mice following a 14-day subcutaneous exposure to Pyridostigmine (PYR). Data is representative of two trials and is represented as mean \pm SEM. Numbers above SEM bars indicate the sample size. $\alpha=0.05$

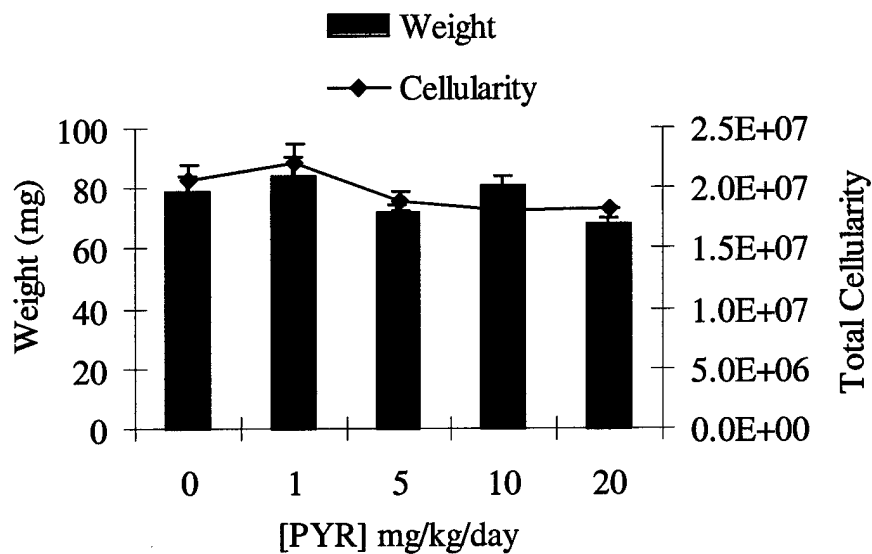


Figure 31. Spleen weights and cellularity in female B6C3F1 mice following a 14-day subcutaneous exposure to Pyridostigmine (PYR). Data is representative of two trials and is represented as mean \pm SEM. Numbers above SEM bars indicate the sample size. $\alpha=0.05$

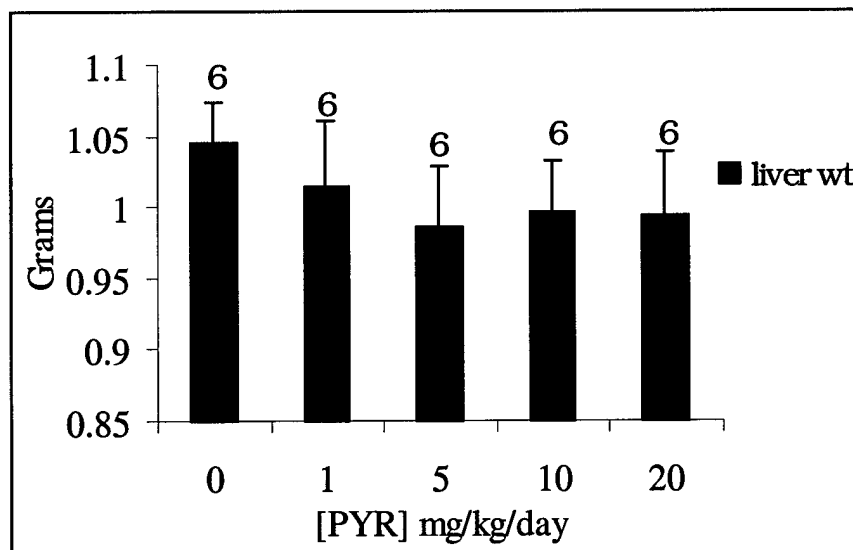


Figure 32. Liver weights in female B6C3F1 mice following a 14-day subcutaneous exposure to Pyridostigmine (PYR). Data is representative of two trials and is presented as mean \pm SEM. Numbers above SEM bars indicate the sample size. $\alpha=0.05$

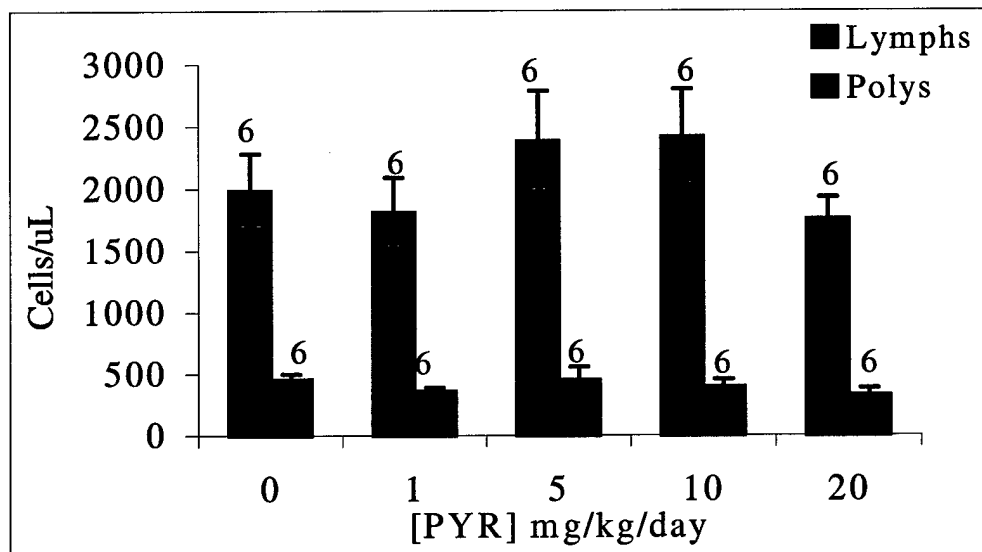


Figure 33. Peripheral blood lymphocyte and PMN counts in female B6C3F1 mice following a 14-day subcutaneous exposure to Pyridostigmine (PYR). Data is representative of two trials and is represented as mean \pm SEM. Numbers above SEM bars are the sample size. $\alpha=0.05$

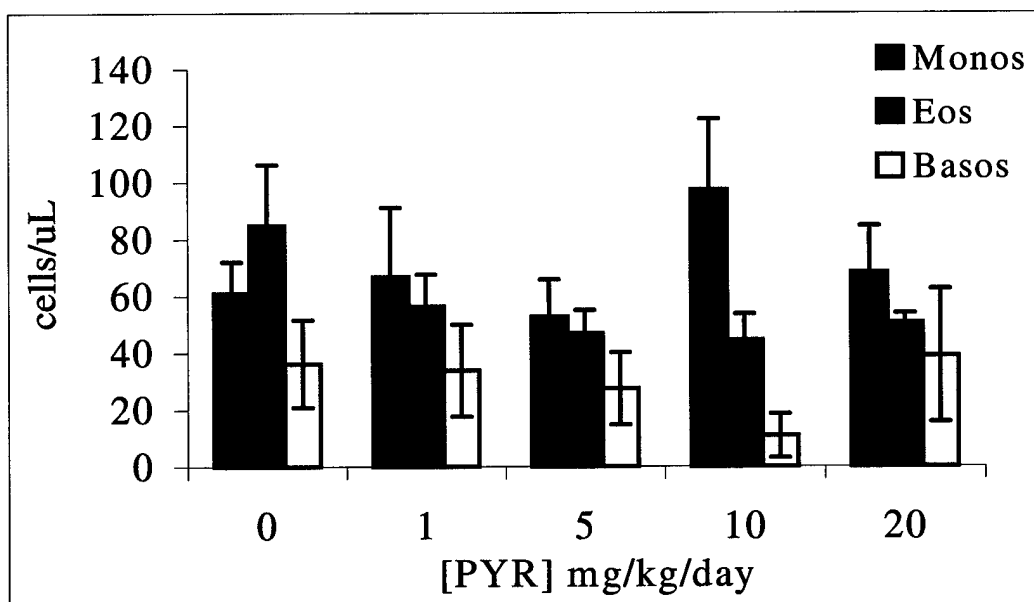


Figure 34. Peripheral blood monocyte, eosinophil, and basophil counts in female B6C3F1 mice following a 14-day subcutaneous exposure to Pyridostigmine (PYR). Data is representative of two trials and is represented as mean \pm SEM. The sample size for all treatments is 6. $\alpha=0.05$

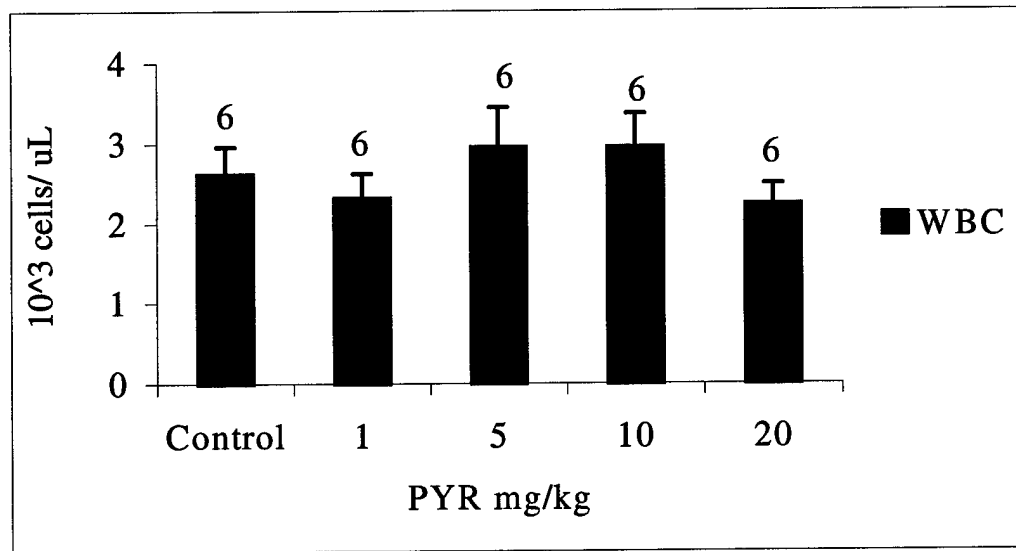


Figure 35. Peripheral white blood cell counts in female B6C3F1 mice following a 14-day subcutaneous exposure to Pyridostigmine (PYR). Data is representative of two trials and is represented as mean \pm SEM. Numbers above SEM bars indicate the sample size. $\alpha=0.05$

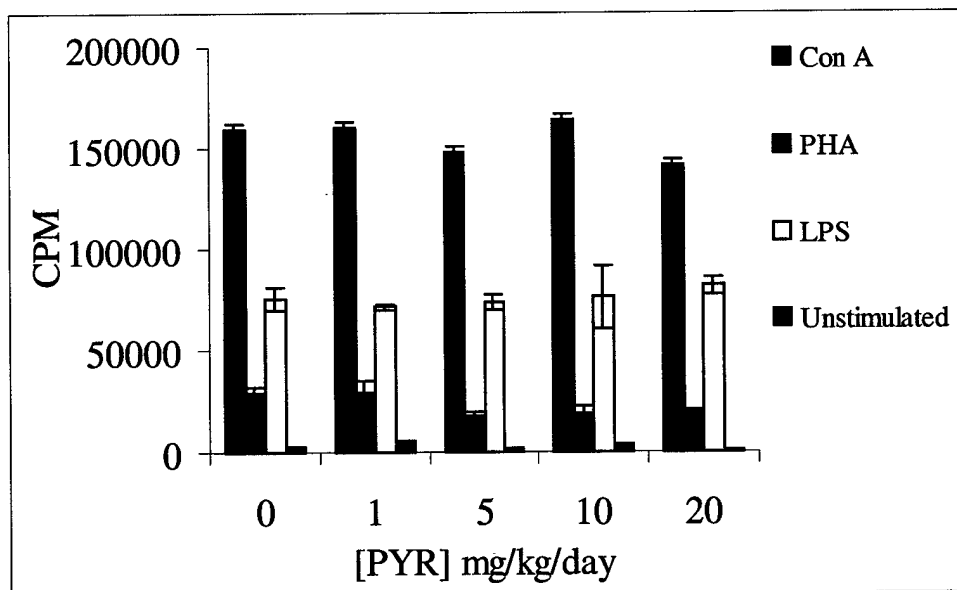


Figure 36. T- and B-cell proliferation in female B6C3F1 mice following a 14-day subcutaneous exposure to Pyridostigmine (PYR). Con A and PHA are T-cell mitogens and LPS is a B-cell mitogen. Unstimulated are wells that did not receive any mitogen. Data is representative of two trials and is presented as mean \pm SEM. Numbers above SEM bars indicate the sample size. $\alpha=0.05$

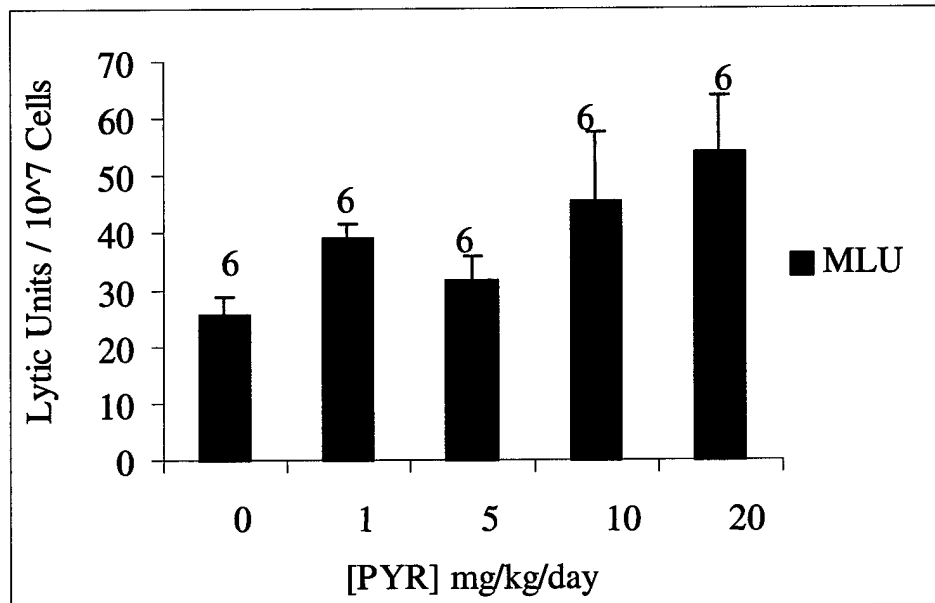


Figure 37. Natural killer cell activity in female B6C3F1 mice following a 14-day subcutaneous exposure to Pyridostigmine (PYR). Data is representative of two trials and is represented as mean \pm SEM. Numbers above SEM bars indicate the sample size. $\alpha=0.05$

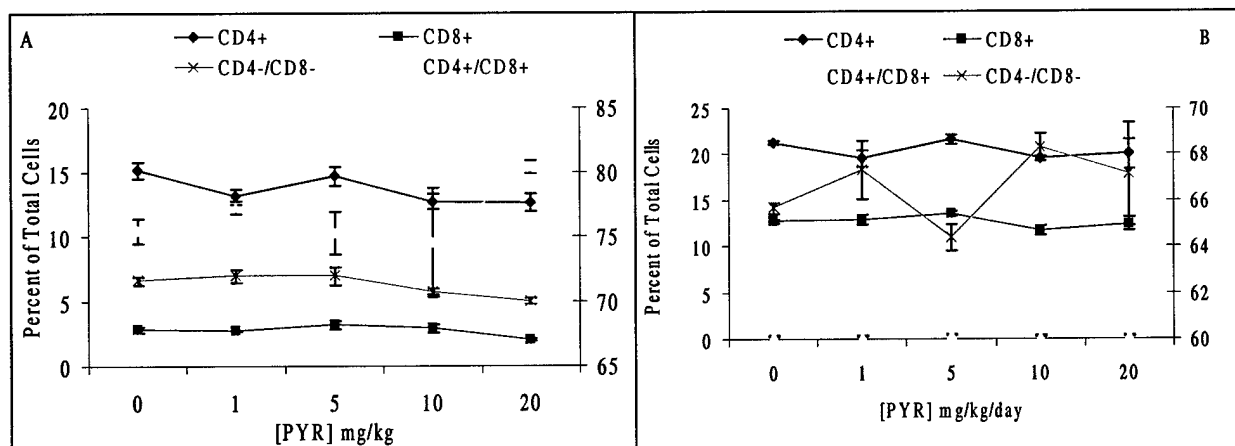


Figure 38. CD4/CD8 subpopulations in female B6C3F1 mice following a 14-day subcutaneous exposure to Pyridostigmine (PYR). Data is representative is from the first trial and is represented as mean \pm SEM. The sample size for all treatment groups is 6, except for the 20 mg/kg/day treatment where the sample size is 3. $\alpha=0.05$. A=Thymic, % CD4+/CD8+ cells are on the secondary axis. B= Splenic, %CD4-/CD8- cells are on the secondary axis.

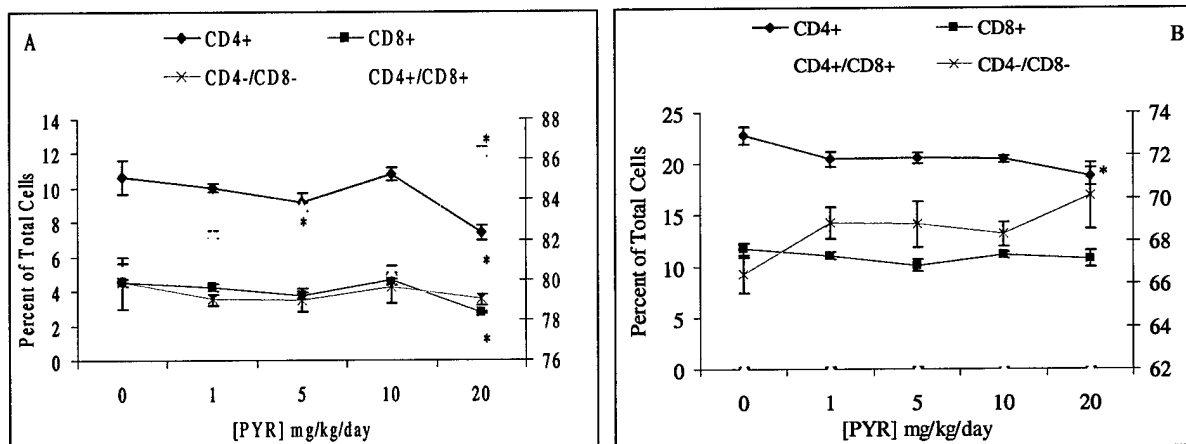


Figure 39. CD4/CD8 subpopulations in female B6C3F1 mice following a 14-day subcutaneous exposure to Pyridostigmine (PYR). Data is representative is from the second trial and is represented as mean \pm SEM. The sample size for all treatment groups is 6. $\alpha=0.05$. A=Thymus; %CD4+/CD8+ are on the secondary axis. B= Spleen; %CD4-/CD8- are on the secondary axis.

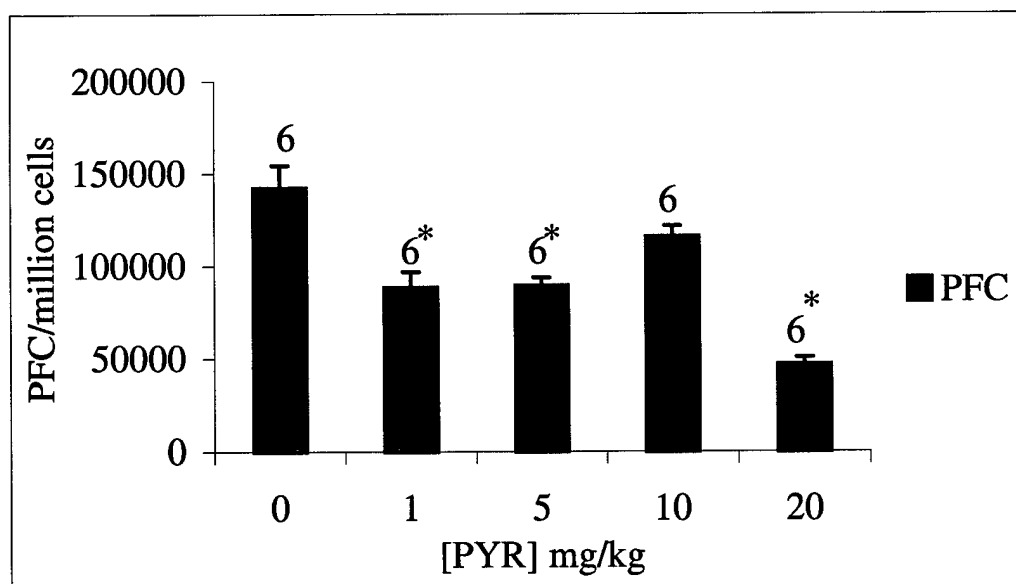


Figure 40. Plaque-forming cell activity in female B6C3F1 mice following a 14-day subcutaneous exposure to Pyridostigmine (PYR). Data is representative of two trials and is represented as mean \pm SEM. Numbers above SEM bars indicate the sample size. $\alpha=0.05$

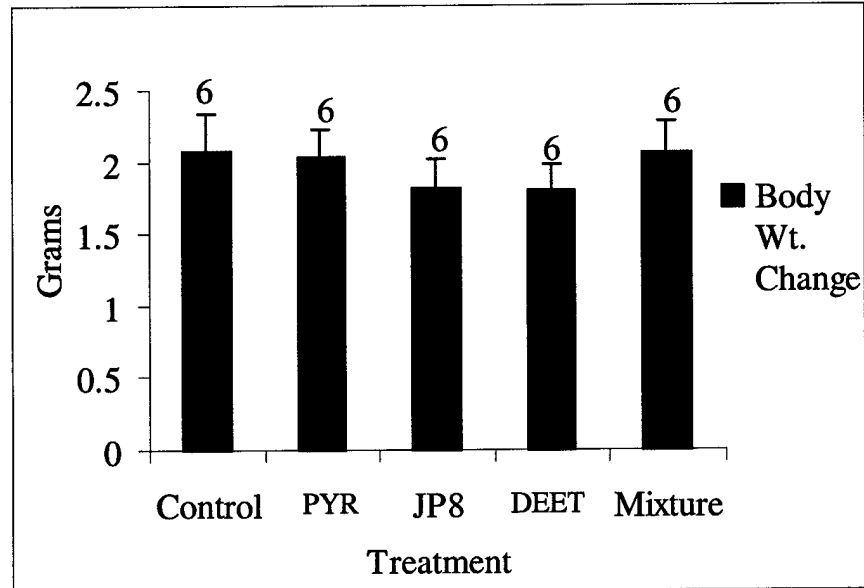


Figure 41. Body weight change in female B6C3F1 mice following 14-day exposure to either 2 mg/kg/day PYR orally, 500 mg/kg/day JP-8 orally, 15.5 mg/kg/day DEET subcutaneously, or exposure to all three concurrently (mixture group). Data is representative of two trials and is presented as mean \pm SEM. Numbers above the SEM bars indicate sample size. $\alpha=0.05$

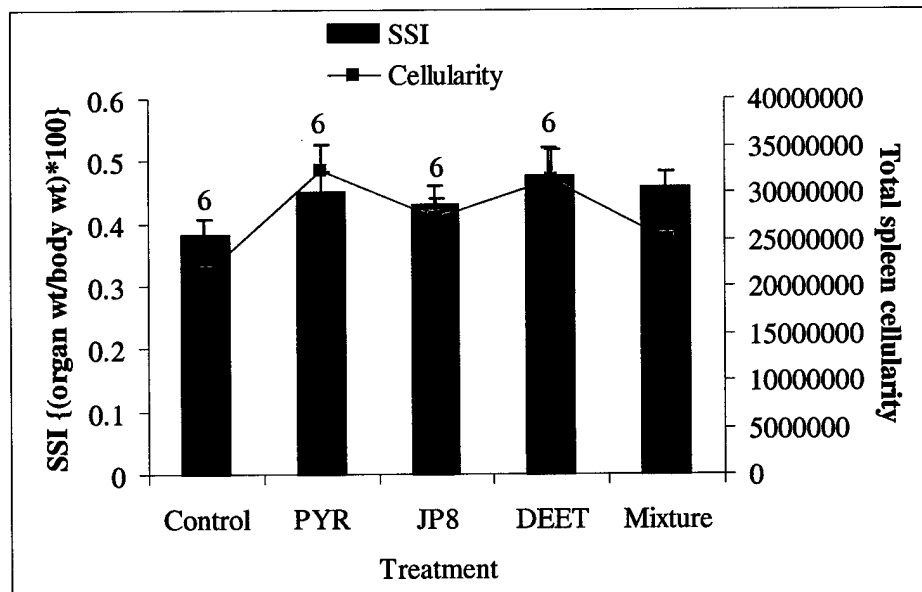


Figure 42. Splenic somatic index (SSI) and cellularity in female B6C3F1 mice following 14-day exposure to either 2 mg/kg/day PYR orally, 500 mg/kg/day JP-8 orally, 15.5 mg/kg/day DEET subcutaneously, or exposure to all three concurrently (mixture group). Data is representative of two trials and is presented as mean \pm SEM. Numbers above the SEM bars indicate sample size. $\alpha=0.05$

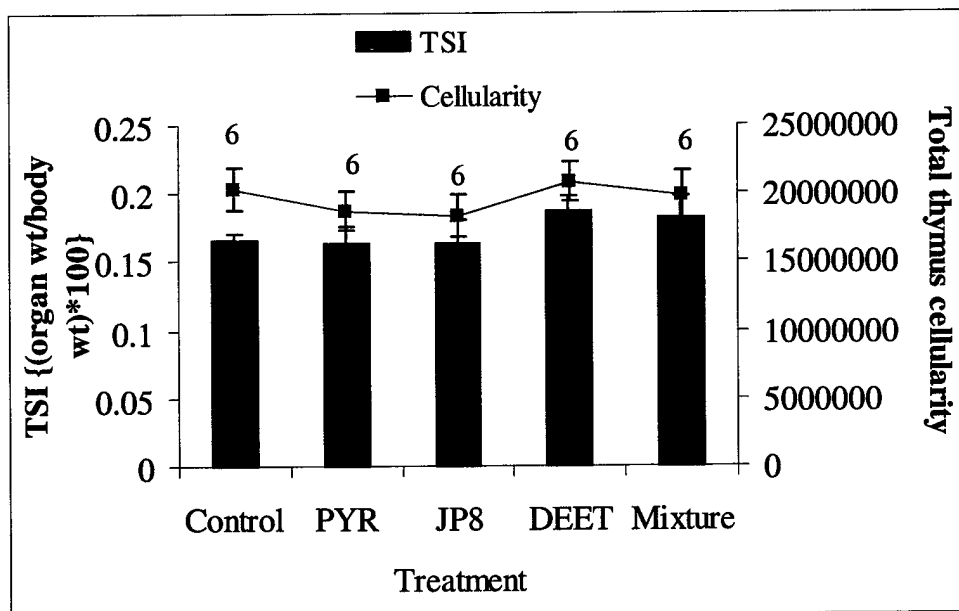


Figure 43. Thymic weight and cellularity in female B6C3F1 mice following 14-day exposure to either 2 mg/kg/day PYR orally, 500 mg/kg/day JP-8 orally, 15.5 mg/kg/day DEET subcutaneously, or exposure to all three concurrently (mixture group). Data is representative of two trials and is presented as mean \pm SEM. Numbers above the SEM bars indicate sample size for both endpoints. $\alpha=0.05$

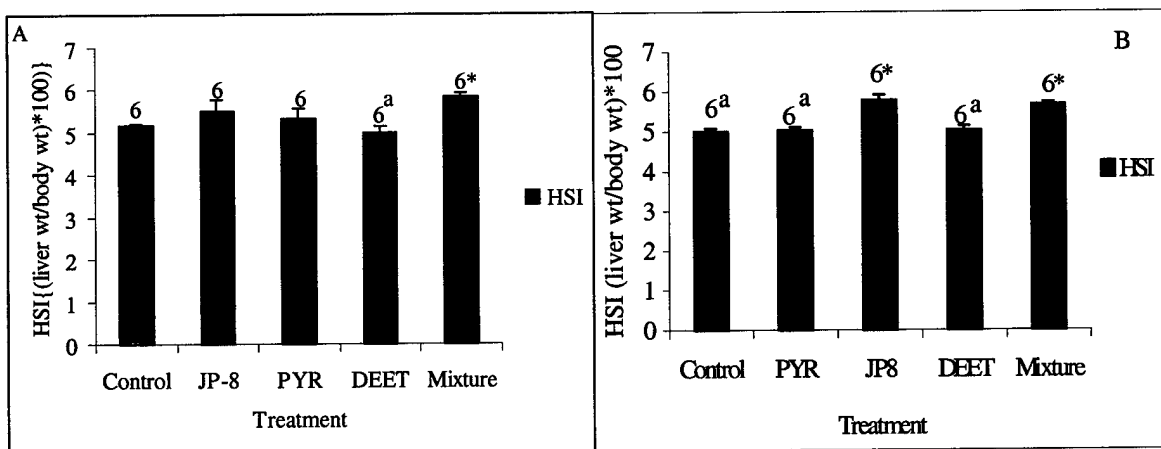


Figure 44. Hepatic somatic index (HSI) in female B6C3F1 mice following 14-day exposure to either 2 mg/kg/day PYR orally, 500 mg/kg/day JP-8 orally, 15.5 mg/kg/day DEET subcutaneously, or exposure to all three concurrently (mixture group). Data is presented as mean \pm SEM. Numbers above the SEM bars indicate sample size for both endpoints. ^{*}Significantly different from control. ^aSignificantly different from the Mixture. $\alpha=0.05$. A=Trials 1, B=Trials 2.

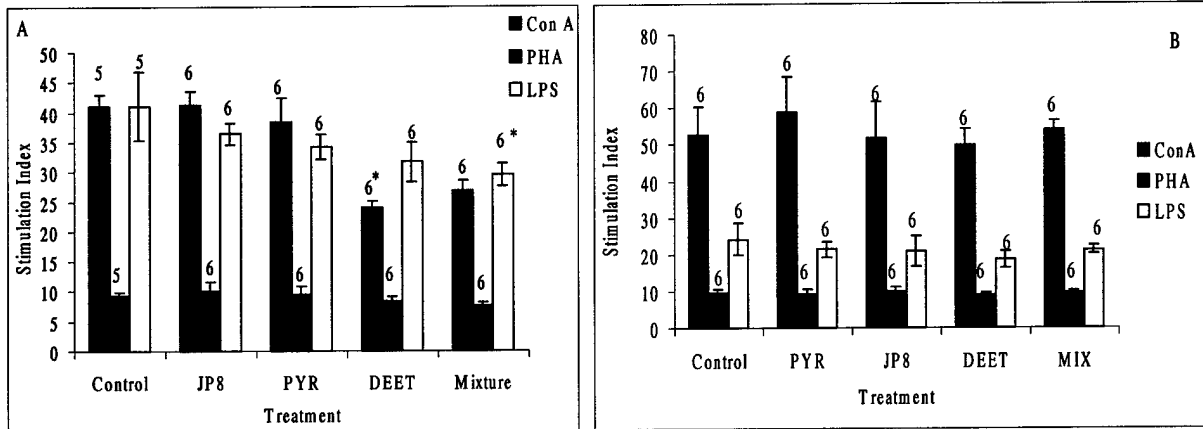


Figure 45. Mitogen induced lymphoproliferation in female B6C3F1 mice following 14-day exposure to either 2 mg/kg/day PYR orally, 500 mg/kg/day JP-8 orally, 15.5 mg/kg/day DEET subcutaneously, or exposure to all three concurrently (mixture group). Data is presented as mean \pm SEM. Numbers above the SEM bars indicate sample size for both endpoints. *Significantly different from control. $\alpha=0.05$. A=Trial, B=Trial 2. Con A=T-cell mitogen, PHA=T-cell mitogen, LPS=B-cell mitogen. Stimulation index = average cpm stimulated/average CPM unstimulated. A=Trial 1, B=Trial 2.

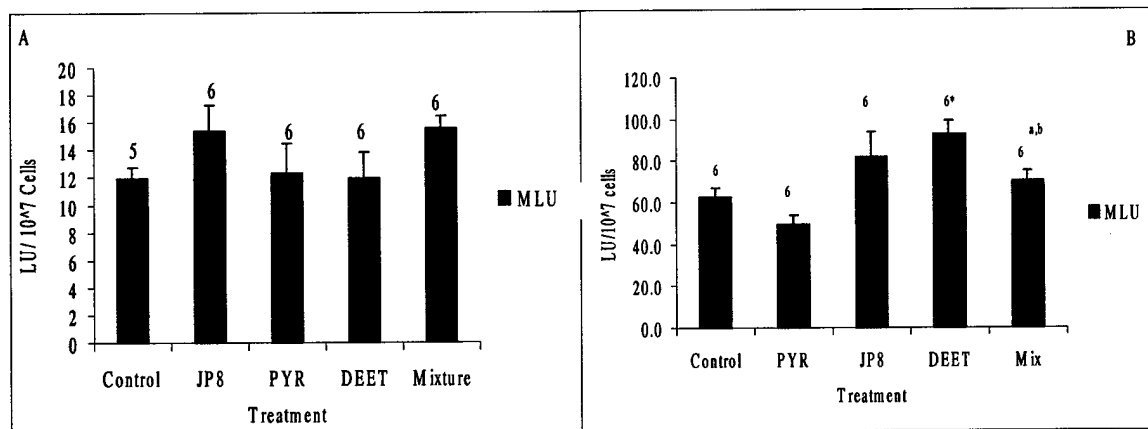


Figure 46. Natural killer (NK) cell activity in female B6C3F1 mice following 14-day exposure to either 2 mg/kg/day PYR orally, 500 mg/kg/day JP-8 orally, 15.5 mg/kg/day DEET subcutaneously, or exposure to all three concurrently (mixture group). Data is presented as mean \pm SEM. Numbers above the SEM bars indicate sample size for both endpoints. *Significantly different from control. ^aSignificantly different from DEET only. ^bSignificantly different from PYR only. $\alpha=0.05$. A=Trial 1, B=Trial 2.

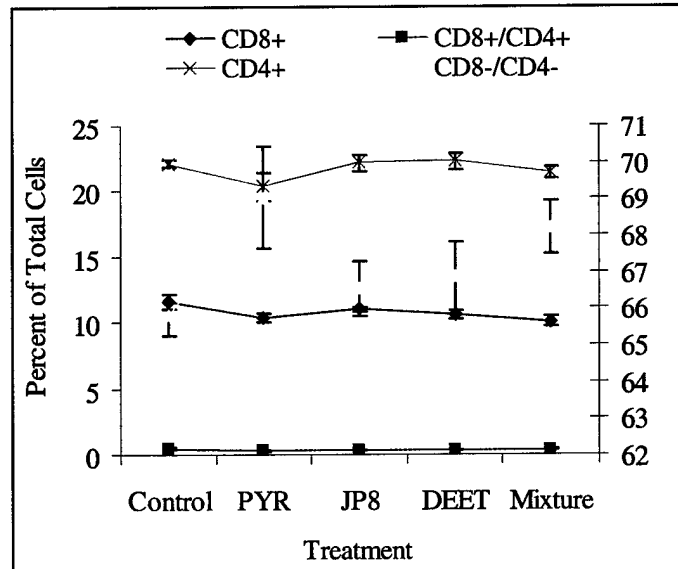


Figure 47. Splenic CD4/CD8 subpopulations percentages in female B6C3F1 mice following 14-day exposure to either 2 mg/kg/day PYR orally, 500 mg/kg/day JP-8 orally, 15.5 mg/kg/day DEET subcutaneously, or exposure to all three concurrently (mixture group). Data is representative of two trials and is presented as mean \pm SEM. Sample size for all treatments is 6. $\alpha=0.05$.

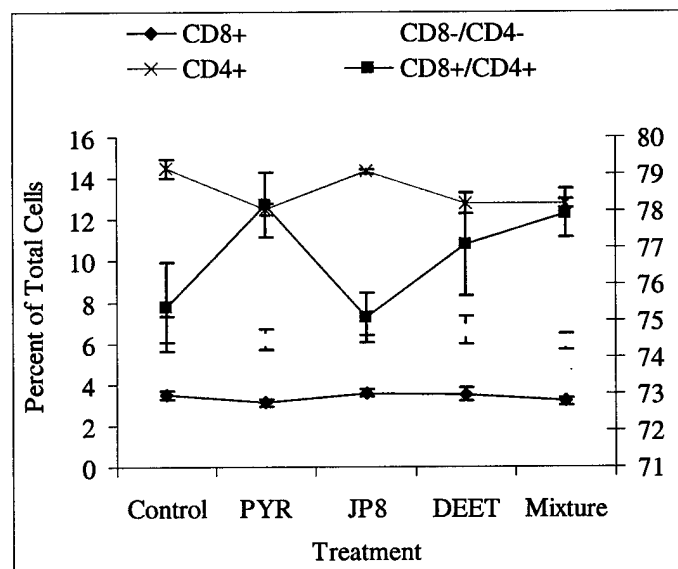


Figure 48. Thymic CD4/CD8 subpopulation percentages in female B6C3F1 mice following 14-day exposure to either 2 mg/kg/day PYR orally, 500 mg/kg/day JP-8 orally, 15.5 mg/kg/day DEET subcutaneously, or exposure to all three concurrently (mixture group). Data is representative of two trials and is presented as mean \pm SEM. Sample size for all treatments is 6. $\alpha=0.05$

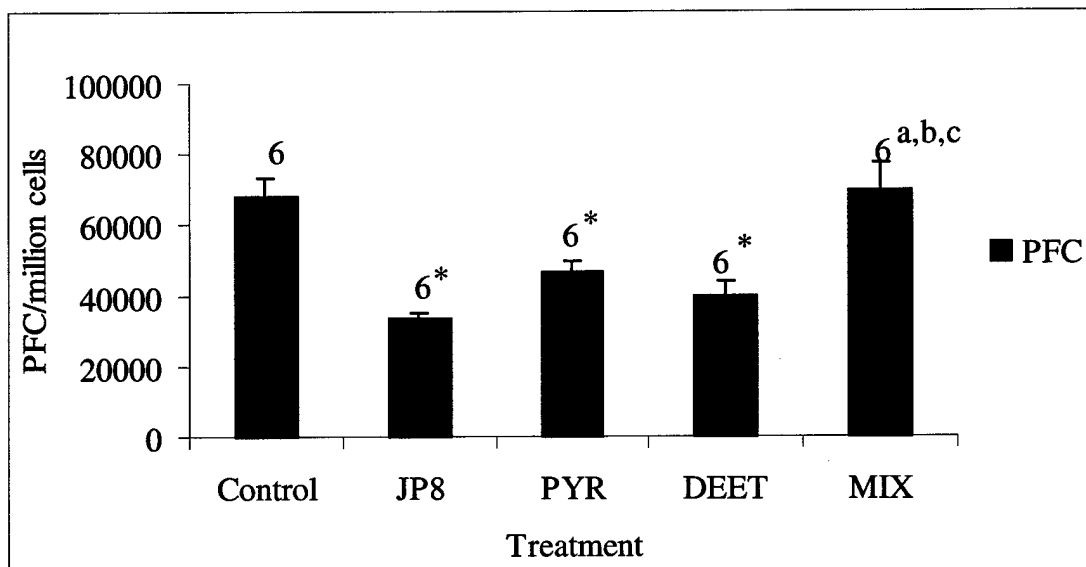


Figure 49. Antibody plaque forming-cell response in female B6C3F1 mice following 14-day exposure to either 2 mg/kg/day PYR orally, 500 mg/kg/day JP-8 orally, 15.5 mg/kg/day DEET subcutaneously, or exposure to all three concurrently (mixture group). Data is presented as mean \pm SEM. Numbers above the SEM bars indicate sample size for both endpoints. *Significantly different from control. ^aSignificantly different from JP-8 only. ^bSignificantly different from PYR only. ^cSignificantly different from DEET only. $\alpha=0.05$

APPENDIX B
Submitted Abstracts

EFFECTS OF *N,N*,*-diethyl-m*-toluamide (DEET) ON IMMUNE FUNCTION PARAMETERS IN B6C3F1 MICE. G S Gilkeson¹, A C Dudley², J G EuDaly³, M M Peden-Adams¹, D E Keil³. ¹Department of Medicine/ Rheumatology and Immunology; ²Department of Environmental Sciences; ³Department of Medical Laboratory Sciences, Medical University of South Carolina (MUSC), Charleston, SC, USA.

It has been suggested that DEET, in combination with a variety of environmental agents relevant to the Gulf War, may have contributed to the manifestations reported by veterans. Since some of the reported symptoms suggest a link to immune dysfunction, it is important to assess the immunological effects of DEET independently and in combination with other environmental agents. To determine the immune effects of DEET exposure singly, adult female B6C3F1 mice were injected subcutaneously for 14 days with DEET only at either 7.7, 15.5, 31, or 62 mg/kg. Effects on lymphoproliferation, natural killer cell activity, thymus and spleen weight and cellularity, and thymic and splenic CD4/CD8 lymphocyte subpopulations were assessed 24 hours after the last dose. No effect was observed in lymphoproliferation, natural killer cell activity, thymic weight, splenic weight, thymic cellularity, or splenic cellularity. Significant decreases were, however, observed in the percentage of thymic CD4-/CD8- lymphocytes at the 15.5, 31, and 62 mg/kg treatment levels and in splenic CD4+ lymphocytes at the 62 mg/kg treatment level. These results suggest that DEET alone does not profoundly affect immune function. Future studies in our laboratory will investigate the immunological and autoimmune effects of DEET in combination with other agents to include JP-8 jet fuel, pyridostigmine bromide, and exercise stress.

EFFECTS OF EXERCISE STRESS OR PYRIDOSTIGMINE BROMIDE (PSB) ON IMMUNE FUNCTION PARAMETERS IN B6C3F1 MICE. M M Peden-Adams¹, A C Dudley², J G EuDaly³, G S Gilkeson¹, D E Keil³. Department of ¹Medicine/Rheumatology and Immunology; ²Environmental Sciences; ³Medical Laboratory Sciences, Medical University of South Carolina, Charleston, SC, USA.

Pyridostigmine bromide (PSB) and physiological stress have been identified as potential agents that contributed to symptoms reported by Gulf War veterans. These exposures in combination with other compounds may result in toxicity to the neuro-endocrine-immune axis thereby leading to the reported manifestations of the Gulf War veterans. To assess the immune effects of these exposures independently, adult female B6C3F1 mice were either gavaged daily for 14 days with PSB (0, 1, 5, 10, or 20 mg/kg) or were challenged daily for 14 days to physiological stress via forced exercise on a treadmill (0, 20, 40, or 60 minutes). Immune parameters assessed were lymphoproliferation, natural killer cell activity, the plaque-forming cell (PFC) response, thymus and spleen weight and cellularity, and thymic and splenic CD4/CD8 lymphocyte subpopulations. No effect was observed in lymphoproliferation or natural killer cell activity following either treatment. Exposure to PSB had no effect on thymic or splenic weights but exercise stress resulted in significant decreases in both spleen and thymus weight at the 40 and 60 minute time points. Both agents significantly decreased the PFC-response (at 1, 5, and 20 mg PSB/kg and at 40 and 60 minutes of exercise, respectively) and altered splenic and thymic CD4/CD8 subpopulations. These studies are a result of our initial efforts to determine the immunological effects due to combined exposure to exercise stress and PSB. Additionally, exposure to these agents will be assessed concurrently with other Gulf War relevant environmental agents such as JP-8 jet fuel and n,n,-diethyl-*m*-toluamide.

An Aryl Hydrocarbon Receptor Independent Mechanism of JP-8 Jet Fuel Immunotoxicity in Ah Responsive (B6C3F1) and Ah Nonresponsive (DBA/2) Mice. Dudley, A.C., Eudaly, J., Peden-Adams, M., Pollenz R.S., and Keil, D. E.

JP-8 jet fuel is a kerosene-based, complex mixture of aliphatic and aromatic hydrocarbons used extensively by the military and commercial airlines. Recent reports have shown that acute, relatively low dose exposures to aerosolized JP-8 vapors can overtly and persistently impair immune function in B6C3F1 mice. Given the evidence that polycyclic aromatic hydrocarbons (PAHs) possess the ability to affect many aspects of the immune system through a putatively described mechanism which involves the aryl hydrocarbon receptor (AhR), it was hypothesized that JP-8 may exert toxicity through AhR-mediated signal transduction. To test this hypothesis, JP-8 was administered by oral gavage for 7 days to either a hydrocarbon 'responsive' strain of mice (B6C3F1) or a classically 'non-responsive' strain (DBA/2) bearing a defective, lower affinity AhR. The results show that each strain was equally sensitive at several endpoints when evaluated for spleen and thymus weight and cellularity, liver weight, B- and T- cell lymphocyte proliferation, and the plaque-forming cell response following *in vivo* JP-8 administration. It was also shown that *in vivo* or *in vitro* JP-8 administration in the murine Hepa - 1 cell line did not induce CYP1A1/2 or promote downregulation of the AhR protein when evaluated by western blot. Conversely, the known AhR ligand 3-methylcholanthrene (3MC) caused a robust upregulation of CYP1A1/2 and downregulation of the AhR in B6C3F1 but not DBA/2 mice and caused a downregulation of the AhR and an increase in CYP1A1/2 *in vitro*. These results support a new hypothesis that JP-8 may exert its toxicity via an AhR-independent mechanism.

APPENDIX C
Curriculum Vitae

Deborah E. Keil, Ph.D.
(formerly D. K. Ensley)

Medical University of South Carolina
Department of Medical Laboratory Sciences
77 President Street, Suite 324
P.O. Box 250702
Charleston, SC, 29425
Tel: (843) 792-3169
Fax: (843) 792-3383
e-mail: keilde@musc.edu

EDUCATION

Ph.D., Biology (Immunotoxicology)	Mississippi State University, MS	1996
B.S., Clinical Laboratory Science	Western Carolina University, NC	1991

PROFESSIONAL EXPERIENCE

Assistant Professor, Department Medical Laboratory Sciences, MUSC	
1996-	
Adjunct Faculty, Marine Biomedical Environmental Science Program, MUSC	1999-
U.S. Army Summer Faculty Research & Engineering Program. Walter Reed Army	
Institute of Research, Washington, D.C. Mentor: Dr. Urszula Kryzch.	1997
Graduate Research/Teaching Assistant, Dept. Biological Sciences, MSU	1991-1996
Medical Technologist, Oktibbeha County Hospital, Starkville, MS	1991-1996
Medical Technologist, Mission Memorial Hospital, Asheville, NC	1991-1991
Counselor, Association for Retarded Citizens, Waynesville, NC	
1988-1991	
Undergraduate Research Assistant, Aquaculture Research Center, WCU, NC	1989-1990

CERTIFICATIONS

Medical Technology, Association of the Society of Clinical Pathologists (MT#188480)	1992-
---	-------

FELLOWSHIPS and HONORS

Developing Scholar of the Year Award, Health Sciences Foundation, MUSC	2000
Scholar of the Year Award, College of Health Professions, MUSC	1999
Outstanding Presentation in Risk Assessment, Society of Toxicology, Baltimore, MD.	1995
Society of Toxicology Travel Award	1995
Department of Biological Sciences Graduate Research Fellowship	1994
Outstanding Presentation, Immunology and Microbiology Division of the	
Mississippi Academy of Science	1992

GRANTS FUNDED

"Immunological Effects of Trichloroethylene Exposure." Continued Funding by Environmental Biosciences Program, Medical University of SC. Total: **\$50,000**. Funding Period: October 1999-October 2000. Primary Investigator: Gary Gilkeson, MD. Co-Investigator: Deborah Keil, Ph.D.

"Immunotoxicity Due to Coexposure of DEET, Pyridostigmine, and Stress." Funded by Department of Veterans Administration. Total: **\$300,000**. Funding Period: November 1998-

November 2001. Primary Investigator: Gary Gilkeson, MD. Co-Investigator: Deborah Keil, Ph.D.

"Evaluation of Immunotoxicity Due to Concurrent Exposure to DEET, Pyridostigmine, and JP-8 Jet Fuel." Funded by the Department of Army. US Army Center for Environmental Health Research. Total: **\$448,369**. Funding Period: February 1999 – February 2002. Primary Investigator: Deborah Keil, Ph.D. Co-Investigator: Gary Gilkeson, MD.

"Intracellular Signaling Pathways as Targets of Uranium Immunotoxicity." Funded by Environmental Biosciences Program, Medical University of SC. Total: **\$47,647**. Funding Period: October 1998- October 1999 (extended). Primary Investigator: Karen Burnett, Ph.D. Co-Investigator: Deborah Keil, Ph.D.

"Immunological Effects of Trichloroethylene Exposure." Funded by Environmental Biosciences Program, Medical University of SC. Total: **\$65,718**. Funding Period: October 1998- October 1999. Primary Investigator: Gary Gilkeson, MD. Co-Investigator: Deborah Keil, Ph.D.

"Effects of Ammonium Perchlorate on Thyroid Hormone Levels, Hematopoiesis, and Immune Status of B6C3F1 Female Mice." Funded by the Department of Defense. US Defense Special Weapons Agency, June 1997. Total: **\$298,177**. Funding Period: January 1998 - August 1999. Primary Investigator: Deborah Keil, Ph.D. Co-Investigator: Alan Warren, Ph.D.

"The Immune Status of Mice Exposed In Utero to JP-8 Jet Fuel: Relationship to Maternal-Fetal Pharmacokinetics." Funded by the Department of Defense. US Defense Special Weapons Agency, June 1997. Total: **\$284,845**. Funding Period: January 1998 - December 1999. Primary Investigator: Deborah Keil, Ph.D. Co-Investigator: Alan Warren, Ph.D.

"Evaluation of a Multivariate Statistical Model for Immunotoxicology Data." Funded by the Dean of the College of Health Professions, Medical University of South Carolina, April 1, 1997. Total: **\$5,000**. Primary Investigator: Deborah Keil, Ph.D.

"Evaluating Immunopotential Provided by Oligofructose: Responses of the Systemic and Enteric Immune Systems to Infectious Pathogens and Cancer Challenges." Funded by ORAFTI, Inc. November 1996. Total: **\$43,500**. Primary Investigator: Randal Buddington, Ph.D. Co-Investigator: Deborah Keil, Ph.D.

BOOK CHAPTERS

Pruett, S.B., and Keil, D.E. 1999. Metam Sodium. Hayes Handbook of Pesticide Toxicology, Academic Press, San Diego. Invited chapter in press.

Wu, W. J., Carson, E. J., Collier, S. D., Keil, D.E., Weiss, P. A., and Pruett, S. B. 1996. Effects of drugs on immune system parameters. Handbook of Human Toxicology. Edited by Dr. E. J. Massaro. CRC Press, Inc., Boca Raton, FL.

Pruett, S.B., Keil, D.E., Ensley, M., and Luebke, R.W. 1996. Interspecies immunotoxicity: Relative importance of acquired and innate immune functions. Modulators of Immune Responses. The Evolutionary Trail. Eds. J.S. Stolen, T.C. Fletcher, C.J. Secombes, J.T. Zelikoff, L.E. Twerdok, D.P. Anderson. SOS Publications, Fair Haven, NJ. p. 343-350

PUBLICATIONS

Peer-Reviewed

Keil, D.E., Luebke, R.W., Ensley, M., Gerard, P., and Pruett, S.B. 1999. Evaluation of multivariate statistical methods for analysis and modeling of immunotoxicology data. *Toxicological Sciences*. 51:245-258

Keil, D.E., Padgett, E.L., Barnes, D.B., and Pruett, S.B. 1996. The role of decomposition products in sodium dimethyldithiocarbamate (SMD)-induced immunotoxicity. *Journal of Toxicology and Environmental Health*, 47:479-492

Moore, C., St. Cyr Coats, K., and Keil, D.E. 1996. Thermal Inactivation of Bovine Immunodeficiency Virus. *Applied and Environmental Microbiology*. 62:4280

Keil, D.E., Luebke, R.W., and Pruett, S.B. 1995. Differences in the effects of dexamethasone on macrophage nitrite production: Dependence on exposure regimen (*in vivo* or *in vitro*) and activation stimuli. *International Journal of Immunopharmacology*, 17:157

Pruett, S. B., Ensley, D.K. and Crittenden, P. 1993. The role of chemical-induced stress responses in immunosuppression: A review of quantitative associations and cause-effect relationships between chemical-induced stress responses and immunosuppression. *Journal of Toxicology and Environmental Health*, 39:163

Educational Reviews

Keil, D.E. 1999. Bacterial sepsis associated with platelet transfusion. *American Society of Clinical Pathologists*. Tech Sample No. I-4. p. 21-26

Keil, D.E. 1998. Serologic problems associated with intravenous immunoglobulin therapy. *American Society of Clinical Pathologists*. Tech Sample No. I-4. p. 23-28

Submitted

Watt, J.M., Holman, S.C., Wilson, W.W., Keil, D.E., Pruett, S.B., and Champlin, F.R. 1999. Influence of serotype A capsulation on cell surface hydrophobicity, charge, and phagocytosis in avian strains of *Pasteurella multocida*. *Microbiology* (resubmitted 1999)

In Progress

Dudley, A.C., EuDaly, J., Peden-Adams, M., Pollenz, R.S., and Keil, D.E. An aryl hydrocarbon receptor independent mechanism of immunotoxicity of JP-8 jet fuel. To be submitted to *Toxicological Sciences*

Keil, D.E., Warren, D.A., Jenny, M.J., EuDaly, J., and Bullard-Dillard, R. Evaluation of thyroid, immunotoxicological, and hematological effects of 14-and 90-day exposures to ammonium perchlorate in B6C3F1 female mice. To be submitted to *Toxicological Sciences*

Keil, D.E., Warren, D.A., Lawrence, J., Bates, T., Jenny, M.J., and EuDaly, J. The immunotoxic effects of JP-8 jet fuel administered by gavage to B6C3F1 female mice. To be submitted to *Toxicological Sciences*

Keil, D.E., Warren, D.A., Lovelace-Robertson, L., Jenny, M.J., EuDaly, J., Lawrence, J., and Kasperski, J. The developmental immunotoxicological effects of *in utero* exposure to JP-8 jet fuel in mice. To be submitted to Toxicological Sciences

POSTERS, ABSTRACTS and PRESENTATIONS

National

Keil, D.E., Warren, D.A., Roberts, L., EuDaly, J., Dudley, A., and Peden-Adams, M. The immunological status of mice exposed *in utero* to JP-8 jet fuel. Presentation at the AFOSR JP-8 Jet Fuel Toxicology Workshop. University of Arizona, Tucson, AZ. Jan 11-12, 2000.

Dudley, A.C., EuDaly, J., Peden-Adams, M., Pollenz, R.S. and Keil, D.E. An Aryl Hydrocarbon receptor independent mechanism of JP-8 jet fuel immunotoxicity in Ah Responsive (B6C3F1) and Ah nonresponsive (DBA/2) mice. Presentation at the AFOSR JP-8 Jet Fuel Toxicology Workshop. University of Arizona, Tucson, AZ. Jan 11-12, 2000.

Keil, D.E., Jenny, M., Warren, D.A., and EuDaly, J. Immune, thyroid and hematological evaluation of ammonium perchlorate in B6C3F1 mice. Accepted to *The Toxicologist*, 2000

Jenny, M., Keil, D.E., Warren, D.A., EuDaly, J. Dietary Iodine modulates ammonium perchlorate induced immunotoxicity. Accepted to *The Toxicologist*, 2000

Gilkeson, G.S., Dudley, A.C., EuDaly, J., Peden-Adams, M.M., and Keil, D.E. Effects of N,N-diethyl-m-toluamide (DEET) on immune function parameters in B6C3F1 mice. Accepted to *The Toxicologist*, 2000

Peden-Adams, M.M., Dudley, A.C., EuDaly, J., Gilkeson, G.S., and Keil, D.E. Effects of exercise stress or pyridostigmine bromide (PSB) on immune function parameters in B6C3F1 mice. Accepted to *The Toxicologist*, 2000

Dudley, A.C., EuDaly, J., Peden-Adams, M.M., and Keil, D.E. An aryl hydrocarbon receptor independent mechanism of JP-8 jet fuel immunotoxicity in two strains of mice. Accepted to *The Toxicologist*, 2000

Jenny, M.J., Keil, D.E., Warren, D.A., Bullard-Dillard, R., and EuDaly, J. Effects of ammonium perchlorate in B6C3F1 mice on thyroid, hematological, and immunological parameters. *The Toxicologist*, Vol. 48, No.1, p.114. March 1999

Junkins, A.D., Hundley, J.M., Sonnett, R.W., Karr, C.K., and Keil, D.E. Development of a clinical practicum course using a simulated clinical laboratory. American Society for Clinical Laboratory Science, San Juan, Puerto Rico. March 1999

Hundley, J. M., Junkins, A.D., Holladay, E.B., Karr, C.K., Sonnett, R.W., and Keil, D.E. An entry level Master of Science degree in Clinical Laboratory Science. American Society for Clinical Laboratory Science, San Juan, Puerto Rico. March 1999

Watt, J. M., Keil, D. E., Pruett, S. B., and Champlin, F. R. Influence of Serotype A encapsulation and experimental decapsulation on phagocytosis by murine peritoneal macrophages in avian isolates of *Pasteurella multocida*. 97th American Society for Microbiology General Meeting, May 1997

Keil, D.E., Luebke, R., and Pruett, S.B. Evaluation of multivariate statistical methods and their application in quantitative modeling of immunotoxicology data. *The Toxicologist*, Vol. 36, No.1, p.200. March, 1997

Watt, J.M., Holman, S.C., Wilson, W.W., Keil, D.E., Pruett, S.B., and Champlin F.R. Effect of experimental decapsulation on cell surface properties in Serotype A *Pasteurella multocida* strains of avian origin. 96th American Society for Microbiology General Meeting, New Orleans, Louisiana, May 1996

Keil, D.E., Ensley, M.D., and Pruett, S.B. Criteria for immunological data for analysis by multivariate methods. *The Toxicologist*, Vol.30, No.1, p.341-342. March 1996

Keil, D.E., Ensley, M.D., Luebke, R.W., and Pruett, S.B. A multi-indicant predictive model of immunosuppression by dexamethasone in B6C3F1 mice. *The Toxicologist*, Vol.15, No.1, p.100. March 1995

Keil, D.E., Luebke, R.W., and Pruett, S.B. Nitrite production of peritoneal macrophages after *in vivo* and *in vitro* exposure to dexamethasone. *The Toxicologist*, Vol.15, No.1, p.176. March 1995

Ensley, D.K., Luebke, R.W., and Pruett, S.B. Quantitative evaluation of the relationship between immune parameters and host resistance. *The Toxicologist*, Vol.14, No.1, p.400. March 1994

Crittenden, P., Padgett, E.L., Ensley, D.K., Carr, R., Burrus, C., and Pruett, S.B. *In vivo* immunotoxicity of methylparathion in B6C3F1 female mice. *The Toxicologist*, Vol.14, No.1, p.123. March 1994

Ensley, D.K., Padgett, E.L., Barnes, D., and Pruett, S.B. Methylisothiocyanate as the primary immunotoxic metabolite of sodium methylthiocarbamate in B6C3F1 female mice. *The Toxicologist*, Vol.13, No.1, p.324. March 1993

Ensley, D.K., Han, Y.C., Padgett, E.L., and Pruett, S.B. Immunotoxicity of a commercial preparation of sodium methylthiocarbamate. *The Toxicologist*, Vol.12, No.1, p.50. February 1992

Regional

Lawrence, J., EuDaly, J., Warren, D.A., and Keil, D.E. Effects of JP-8 jet fuel on hematopoiesis in B6C3F1 mice. Carolina's Clinical Connection Meeting. March 1999

Marquis, T., Jenny, M.J., Warren, D.A., and Keil, D.E. Effects of JP-8 jet fuel on macrophage function in B6C3F1 mice. Carolina's Clinical Connection Meeting. March 1999

Keil, D.E. Invited Speaker. "Malaria - Development of a Vaccine." Carolinas Clinical Connection. Charlotte, NC. 1998

Keil, D.E. Invited Speaker. "Drugs and their Effect on the Immune System" Tennessee State Society of American Medical Technologists, Continuing Education Seminar and State Meeting, Nashville, TN. 1995

Ensley, D.K., Luebke, R.W., and Pruett, S.B. Quantitative relationships between immunological parameters and host resistance: A multivariate statistical approach. Presentation at the Eighth Annual Symposium on Immunotoxicology, Virginia Beach, VA. September 1993

Ensley, D.K., Padgett, E.L., Barnes, D., and Pruett, S.B. The effects of methylisothiocyanate, the primary immunotoxic metabolite of sodium methyldithiocarbamate. Regional Society of Microbiology and Biochemistry, Starkville, MS. November 1992

Ensley, D.K., Han, Y.C., Padgett, E.L., and Pruett, S.B. An investigation of the immunotoxic effects of sodium methyldithiocarbamate in B6C3F1 female mice. South Central Chapter of the Society of Toxicology, Starkville, MS. September 1991

Pool, G.L., Ensley, D.K., Samples, B.L., and Lumb, R.H. PAF stimulation of the respiratory burst in phagocytes of the rainbow trout. Southeastern Regional Lipid Conference, Cashiers, NC. October 1990

University

Jenny, M.J., Keil, D.E., and EuDaly, J. Dietary iodine alters suppression of thyroid hormone biosynthesis and ammonium perchlorate induced immunotoxicity. 34th Annual MUSC Student Research Day. November 1999

Dudley, A.C., EuDaly, J., Peden-Adams, M.M., and Keil, D.E. An aryl hydrocarbon receptor independent mechanism of JP-8 jet fuel immunotoxicity in two strains of mice. 34th Annual MUSC Student Research Day. November 1999

Jenny, M.J., Keil, D.E., Warren, D.A., Bullard-Dillard, R., and EuDaly, J. Effects of ammonium perchlorate in B6C3F1 mice on thyroid, hematological, and immunological parameters. 33rd Annual MUSC Student Research Day. November 1998

BOOK REVIEW

Medical Laboratory Management and Supervision: Operations, Review, and Study Guide. By Lionel A. Varnadoe. 1997. Reviewed by D. Keil. Journal of Allied Health. 26:142

TEACHING/MENTORING EXPERIENCE

Theses Completed

1999	Matthew Jenny, M.S.	The effects of ammonium perchlorate on thyroid, hematological and immunological function in B6C3F1 female mice
1999	Jessie Lawrence, M.S.	The effects of JP-8 jet fuel on hematopoiesis in B6C3F1 mice
1999	Tracy Marquis, M.S.	The effects of JP-8 jet fuel on macrophage function in B6C3F1 mice
1999	Julie Kasperski, M.S.	Comparative study on the cytokine levels in irradiated and filtered platelet concentrates

Anticipated

2000	Andrew Dudley, M.S.	Effects of concurrent exposure to DEET, pyridostigmine, and exercise stress on immunological parameters
2000	Camilla Allen, M.S.	The effects of ellagic acid on natural killer cell activity and B16F10 tumor resistance
2000	Kimberlee Banks, M.S.	The effects of concurrent exposure of DEET, pyridostigmine

2000	Stacey Wallace, M.S.	bromide, and JP-8 on humoral antibody responses The effects of trichloroethylene on macrophage function in NZB/NZW and B6C3F1 mice
2000	Nikki Hughes, M.S.	Use of glucagon in the treatment of carbon monoxide poisoning

Undergraduate and High School Mentoring

1999	Tameka Greene	McClellenville High School, SC
1998	Nina Montgomery, BS	Claflin College, Orangeburg, SC
1998	Charlease Kelly, BS	Claflin College, Orangeburg, SC

Courses Taught in Clinical Laboratory Sciences Masters Program, MUSC

Professional Practice in Immunohematology	1998-
Topics in Immunohematology	1998-
Research and Statistics	1998-
Immunohematology and Transfusion Practices	1997-
Introduction to Laboratory Management	1997-

Courses Taught as a Graduate Assistant, Mississippi State University

Medical Parasitology	1992-1996
Quantitative Methods in Medical Technology	1992-1996
Botany	1991-1993

SERVICE**University**

University Gender Issues Committee	1999-
Electronic Research Administration Working Group	1998-
Basic Science Building Science Advisor	1998-
Operations Committee, Strom Thurmond Biological Research Building	1998-
University Research Council	1998-
Faculty Senate, MUSC	1998-

College

Search Committee for Assistant Dean of Research	1999-
Research Task Force, College of Health Professions	1998-
Chair of the Research Subcommittee, College of Health Professions	1997-

Department

Marine Biomedical Environmental Science Committee	1998-
Departmental Recruitment Committee	1997-
Departmental Safety Committee	1997-

SOCIETY MEMBERSHIP

South Carolina Academy of Science	1999-
American Society of Clinical Laboratory Science	1997-
Associate Membership with the Society of Toxicology	1997-
Association for the Society for Clinical Pathologists (ASCP)	1992-

CONTINUING EDUCATION

AFOSR JP-8 Jet Fuel Toxicology Workshop. Tucson, AZ	2000
WebCT Course, MUSC	1999
Conference on Federally Sponsored Gulf War Veterans' Illnesses Research Pentagon City, VA	1999
Workshop on Human and Murine Stem Cell Evaluation. Stem Cell Technologies, Inc. Vancouver, BC	1999
38 th Annual Society of Toxicology Meeting, New Orleans, LA	1999
Linking Environmental Agents and Autoimmune Diseases. National Institute of Environmental Health Sciences. Research Triangle Park, NC	1998
Assessing and Managing Risks in a Democratic Society. Society for Risk Analysis Annual Meeting and Exposition, Phoenix, AZ.	1998
36 th Annual Society of Toxicology Meeting. Cincinnati, OH	1997
35 th Annual Society of Toxicology Meeting, Anaheim, CA	1996